(19) World Intellectual Property Organization International Bureau



(81) Designated States (national): AE, AG, AL, AM, AT, AU,

(43) International Publication Date 3 July 2003 (03.07.2003)

(51) International Patent Classification7:

C12N

(10) International Publication Number WO 03/054162 A2

(22	International Application Number: PCT/US02/41014 International Filing Date: 19 December 2002 (19.12.2002)	AZ, BA, BB, BC, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, SH, FL, BG, D, GE, GH, GM, HR, HU, ID, H, N, IS, JP, KR, KG, KP, KR, KZ, LC, LK, IZ, LS, LT, LI, UJ, WA, MD, MG, MK, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TJM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.
(25	Filing Language: English	
•	Publication Language:	(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BI, CF, CG, CI, CM, GA, GN, GG).
•	Applicant (for all designated States except US): AMBION, INC. [US/US]; 2130 Woodward St., Suite 200, Austin, TX 78744-1832 (US).	GW, ML, MR, NE, SN, TD, TG). Published: - without international search report and to be republished upon receipt of that report
(72) (75)	Inventors; and Inventors/Applicants (for US only): MURPHY, George, L. [US/US]; 8225 Escabosa Dr., Austin, TX 78748 (US). WHITLEY, J., Penn [US/US]; 5203 Avenue F, Austin, TX 78751 (US).	For two-letter codes and other abbreviations, refer to the "Guid- ance Notes on Codes and Abbreviations" appearing at the begin- ning of each regular issue of the PCT Gazette.
(72) (75) (74)	Ageat: SHISHIMA, Giaa, N.; Fulbright & Jaworski LLP, Suite 2400, 600 Congress Avenue, Austin, TX 78701 (US).	

(54) Title: METHOD AND SYSTEM FOR DEPLETING IRNA POPULATIONS

(57) Abstract: The present invention concerns a system for isolating, depleting, or separating a targeted nucleic acid, such as rRNA. from a sample comprising targeted and nontargeted nucleic acids. It effects a way of enriching for nontargeted nucleic acids, such as mRNAs. The invention further concerns methods of implementing the system and kits for implementing the system, which involves at least one bridging nucleic acid comprising 1) a targeting region complementary to a region on the targeted nucleic acid and 2) a bridging region complementary to the capture region of a capture nucleic acid that comprises a nonreactant structure. The nonreactant structure can be used to isolate the hybridizing molecules after incubation under conditions that allows hybridization.

WO 03/054162 PCT/US02/41014

DESCRIPTION

METHOD AND SYSTEM FOR DEPLETING rRNA POPULATIONS

BACKGROUND OF THE INVENTION

Field of the Invention

5

10

15

20

25

30

The present invention relates generally to the fields of molecular biology and microbial pathogenesis. More particularly, it concerns methods, compositions, and kits for isolating, depleting, separating a targeted nucleic acid population from other nucleic acid populations as a means for enriching those other nucleic acid population(s). More particularly, it concerns methods, compositions, and kits for enriching mRNA populations by depleting eukaryotic and/or prokaryotic rRNA from a sample using engineered bridging and capture nucleic acid molecules.

2. Description of Related Art

The ongoing efforts in microbial genome sequencing will enable unprecedented advances in our understanding of microbes and host-microbe interactions. Dozens of prokaryotic genomes, including those of numerous human pathogens, have been completely sequenced, and many others are in progress. Consequently, a renewal of focus and energy has emerged in the fields of microbial evolution, microbial pathogenesis, and infectious diseases. The potential impact of genomics on these disciplines is the subject of several recent reviews (Cummings et al., 2000; Cornelis et al., 2001; Fox et al., 2001; Current Opinion in Microbiology). For host-microbe interactions, the ability to measure the expression of every single gene in a microorganism will make possible studies of such complex interactions as the global regulation of virulence factors and the mechanisms of response to host cells and their microenvironment. Scientists will also be able to evaluate the complete repertoire of host cell gene expression in response to the pathogen. Undoubtedly, novel interactions and responses between microbes and their hosts will be discovered, leading to a more complete picture of infectious diseases and how to control them.

In the past decade, researchers studying bacteria developed several novel approaches to evaluate global gene transcription in response to environmental stimuli, including host-microbe interactions. Prior to the era of genome sequencing, Chuang et al. (Chuang et al., 1993) used an ordered set of E. coli lambda library clones to evaluate global transcription responses of E. coli. Other groups employed subtractive hybridization and differential screening to evaluate induction

of gene expression in Mycobacterium avium after phagocytosis by macrophages (Plum et al., 1994) or in Pyrococcus grown under specific environmental conditions (Robinson et al., 1994). Researchers further developed this approach with an elegant procedure for the selective capture of transcribed sequences (SCOTS) (Graham et al., 1999). At the same time, many scientists bypassed library construction altogether and used using differential display (Liang et al., 1995) to discover genes that are transcribed differently under various growth conditions. Although useful in certain circumstances, differential display is frequently a hit-or-miss prospect and gives no information on global transcription. More recently, serial analysis of gene expression (SAGE) (Velculescu et al., 1995) emerged as a method for analyzing the complete transcriptome of a cell. SAGE, like differential display, can be useful but requires large amounts of nucleic acid sequencing. Not unexpectedly, for organisms whose genomes have been sequenced, array analysis is emerging as the method of choice for global gene expression studies with bacteria. Macroarrays (filter-based arrays) and microarrays (slide-based arrays) of complete genomes have made possible the simultaneous expression analysis of thousands of genes. The advent of microarray technology has already enabled analyses of the host response to interactions with pathogenic organisms (Cummings et al., 2000). Similarly, microarray analysis and other methods have been used to evaluate gene expression in bacteria grown under different environmental conditions in vitro.

10

20

25

30

The application of array analysis to gene expression profiling in prokaryotes was an immediate outgrowth of similar studies with eukaryotic organisms, occurring only within the past two to three years. Infectious disease researchers have already begun applying microarray analysis to the study of complex host-microbe interactions. To date, such analyses of host-microbe interactions have been limited to the evaluation of host cell responses to bacteria or viruses. Bordetella pertussis, Listeria monocytogenes, Neisseria meningitidis, Pseudomonas aeruginosa, Legionella pneumophila, Salmonella dublin, and Staphylococcus aureus are among the bacterial pathogens whose effects on host cell gene expression have been evaluated with microarrays. Array analyses of eukaryotic host cell transcription are feasible because of the ability to isolate polyadenylated mRNAs from eukaryotic cells and to specifically label mRNAs by oligo dT-primed cDNA synthesis.

Although it has been alluded to in the literature (Cummings et al., 2000; Rappuoli, 2000), complete genome array expression analyses of bacteria in response to interactions with host cells

have not been widely published, if at all. Studies that examine the global bacterial gene response in the presence of host cells will require the development of tools to enable the efficient isolation, enrichment, and labeling of bacterial mRNAs (Cummings et al., 2000; Graham et al., 1999; Gingeras et al., 2000; Graham et al., 2001).

However, technical limitations of current methods available for purification and evaluation of bacterial mRNAs preclude these types of whole genome analysis. To realize the full potential of the genomics revolution, methods for purifying mRNAs from total bacterial RNA populations and particularly from mixtures of host cell and bacterial RNA need to be developed.

5

10

15

25

30

Isolating sufficient quantities of high quality bacterial mRNA is perhaps the most demanding technical requirement impeding analyses of bacterial gene expression in the presence of host cells. A small percentage of bacterial mRNAs may be A-tailed, but these are targeted for degradation and tend to be unstable. As a result, the commonly used method for mRNA purification with eukaryotic cells. oligo-dT capture, is ineffective.

Only a few studies have described methods for enriching or purifying bacterial mRNAs. Several groups (Plum et al., 1994; Robinson et al., 1994; Su et al., 1998) have used rRNA subtraction to enrich for bacterial mRNAs. These procedures involved hybridization of rRNAs to biotinylated plasmid containing rRNA genes or to biotinylated antisense rRNAs followed by streptavidin capture and removal. This yields some benefits, but it requires fairly large amounts of plasmids or antisense RNA. Biotinylation of large amounts of DNA or RNA is often tricky and can be prohibitively expensive if biotin-modified nucleotides are incorporated during antisense RNA synthesis. In general, these methods have not seen widespread use. As mentioned above, Graham and Clarke-Curtiss (Graham et al., 1999) went further in enriching for mycobacterial mRNAs with SCOTS. The SCOTS procedure is effective for detecting genes specifically expressed in the presence of host cells but is hampered by being a multi-step procedure that requires production of normalized double-stranded cDNA, PCR, differential hybridization, and cDNA capture. In addition to these methods, researchers have developed methods to polyadenylate bacterial mRNAs, thereby allowing for their purification by oligo dTcapture. Amara and Vijaya (Amara et al., 1997) demonstrated that mRNAs in purified polysomes can be specifically polyadenylated and purified by oligo-dT capture. Wendisch et al. (Wendisch et al., 2001) showed that the same process can be carried out with crude cell extracts.

Several shortcomings are associated with the polyadenylation approach. Different mRNAs may be polyadenylated to different extents or not at all depending on the structure of their 5' and 3' ends (Feng et al., 2000). Polyadenylation in a cell lysate, followed by purification of RNA, will require inactivation of cellular RNAses so that transcripts are not degraded during the polyadenylation reaction. Optimizing the reaction to work reproducibly in many different bacterial cell lysates would likely be very difficult. Despite many worthy attempts, simple and universal procedures for bacterial mRNA enrichment, especially in the presence of host cell RNA, remain elusive. Thus, there remains a continued need for improvements in mRNA enrichment and/or the depletion of other RNA populations.

SUMMARY OF THE INVENTION

10

15

20

25

30

The present invention involves a system that allows for the isolation, separation, and depletion of a population of nucleic acid molecules. The system involves components that may be used to implement methods for isolating, separating, or depleting a targeted nucleic acid. Such components may also be included in kits of the invention.

In embodiments of the invention, a population of nucleic acids may be targeted for isolation, separation, or depletion. Such a nucleic acid is referred to as "targeted nucleic acid" or "targeted nucleic acid molecule." Alternatively, it may be referred to as a "nucleic acid target." In particular embodiments of the invention, the targeted nucleic acid is rRNA. In alternative embodiments, the targeted nucleic acid is mRNA, tRNA, or DNA including, cDNA and genomic DNA. The targeted nucleic acid may be in a sample, which is a composition that is suspected of containing the targeted nucleic acid. In some embodiments, the sample is obtained from or includes prokaryotes or eukaryotes or both. The sample may be cells, tissues, organs, and lysates, fractionations, or portions thereof. Furthermore, the targeted nucleic acid is targeted via a "targeting region" in the targeted nucleic acid. A "targeted region" refers to a region of the targeted nucleic acid that is complementary with the targeting region of a bridging nucleic acid and that allows the targeted nucleic acid to be separated from other non-targeted nucleic acid populations.

In embodiments in which the targeted nucleic acid is rRNA, the rRNA may be the 5S, 16S, or 23S rRNA from prokaryotes, though it may be any rRNA species from a prokaryotes. It is specifically contemplated that nucleic acids may be targeted in Gram positive bacteria and Gram negative bacteria. In further embodiments, the targeted rRNA is 5.8S, 17S or 18S, or 28S

rRNA (referred to as "types of rRNA") from a eukaryote. It is further contemplated that tRNA may be a targeted nucleic acid population either by itself or in combination with any of the targeted nucleic acids described herein. A non-limiting list of targeted rRNAs from various organisms is provided in a later section and is contemplated to be part of the invention.

5

10

15

20

25

30

In embodiments of the invention, the system involves a bridging nucleic acid, a capture nucleic acid, and a targeted nucleic acid, as shown, for example, in FIG. 1. While in many embodiments of the invention it is contemplated that the bridging nucleic acid and the capture nucleic acid are oligonucleotides, it is specifically contemplated that they may be polynucleotides as well. Thus, any embodiment involving an oligonucleotide may be implemented with a polynucleotide. Bridging nucleic acids, capture nucleic acids, and targeted nucleic acids of the invention may include, be at least or be at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66. 67. 68. 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310 320, 330, 340, 350, 360, 370, 380, 390, 400, 410. 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000 or more residues in length.

Furthermore, a "bridging nucleic acid" is a nucleic acid molecule that comprises a bridging region and a targeting region, while a "capture nucleic acid" is a nucleic acid molecule that comprises a capture region. It is contemplated that bridging, targeting, and capture regions of the invention may be, be at least or be at most 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830,

840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, or 1000 residues in length.

A "bridging nucleic acid" refers to a molecule that includes nucleic acid residues or analogs and that includes at least one targeting region and at least one bridging region. A "targeting region" refers to a region of the molecule that is involved in targeting a particular nucleic acid or nucleic acid population and is thus complementary to all or part of the sequence of the targeted nucleic acid. It is further contemplated that more than one targeting region may be included in a bridging nucleic acid. The bridging nucleic acid may include or have up to 2, 3, 4, 5, 6, 7, 8, 9, 10, or more targeting regions. When there are multiple targeting regions, it is contemplated that the regions may be complementary to different, nonoverlapping sequences from the same targeted nucleic acid or they may be complementary to similar or overlapping sequences from the same targeted nucleic acid, or they may be complementary to sequences in different targeted nucleic acids. While mRNA may be targeted, it is specifically contemplated that mRNA is not targeted and thus the targeting region does not have a stretch of polypyrimidine residues, such as poly-T or poly-U to hybridize to the poly-A tail of eukaryotic mRNA. Also considered part of the invention is using single or multiple bridging nucleic acids to deplete an rRNA population. In some embodiments, a single bridging nucleic acid may contain one or more targeting regions that are complementary to different types of rRNA ("types" refer to sizes based on intact lengths). Thus, in some embodiments, the largest type of rRNA may be targeted ("largest" refers to longest nucleic acid molecule when intact, even though molecules that are no longer intact may also be targeted if they retain the sequence that is complementary to all or part of a targeting region). In still further embodiments, the second largest rRNA or the first and second largest rRNA types may be targeted by a single bridging nucleic acid with targeting regions to each or to more than one nucleic acid, each with a targeting region to a different type of rRNA. In still further embodiments, a bridging nucleic acid has a targeting region complementary to one or more of the following prokaryotic and eukaryotic rRNA types: 5S, 16S, 23S, 5.8S, 17S, 18S, and/or 28S. A bridging nucleic acid may target 1, 2. 3, 4, 5, 6, 7, or more types of rRNA, as well as any and all tRNA types, both eukaryotic and prokaryotic.

10

15

20

25

30

A "bridging region" in a bridging nucleic acid refers to a region that mediates an interaction with a capture nucleic acid. In further embodiments, the bridging region is a

polypurine or polypyrimidine stretch of residues. A bridging region can include a stretch of adenine or guanine residues or cytosine, uracil, or thymidine residues. In some embodiments, it is contemplated that more than one bridging region is included in a bridging nucleic acid, such as 2, 3, 4, 5, or more bridging regions.

5

10

20

25

30

A "capture nucleic acid" refers to a molecule that includes nucleotides or nucleotide analogs, a capture region, and a nonreacting structure. A "capture region" refers to a region that interacts with the bridging region of a bridging nucleic acid. In embodiments of the invention, the bridging region and the capture region are complementary to each other and hybridize to one another under conditions that allow for hybridization of complementary regions. In some embodiments of the invention, the capture region and bridging region are a stretch of complementary repeated nucleotides (complementary homopolymeric regions). For example, they may be homopolymeric A, T, G, C, or U. In other embodiments of the invention, however, the bridging and capture regions are any sequence, so long as they are complementary. In some embodiments of the invention, the capture region has a sequence that includes at least 5, 6, 7, 8, 9, 10 or more contiguous nucleotides of SEQ ID NO:87, SEQ ID NO:88, SEQ ID NO:91, and SEQ ID NO:92 (collectively referred to as "SEQ ID NO:88, 252). In some embodiments, the capture regions comprises any of of SEO ID NOs 87-92.

There may be more than one nonreacting structure attached, covalently or noncovalently, to a capture nucleic acid. There may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more nonreacting structures as part of a capture nucleic acid.

A capture nucleic acid also includes a "nonreacting structure," which refers to a compound that does not chemically react with a nucleic acid. In some embodiments, a nonreacting structure is a magnetic bead or rod, which allows the capture nucleic acid, a bridging nucleic acid and a target nucleic acid to be isolated from a sample with a magnetic field, such as a magnetic stand. In still further embodiments, the nonreacting structure is a bead or other structure that can be physically captured, such as by using a basket, filter, or by centrifugation. It is contemplated that a bead may include plastic, glass, teflon, silica, a magnet or be magnetizeable, a metal such as a ferrous metal or gold, carbon, cellulose, latex, polystyrene, and other synthetic polymers, nylon, cellulose, nitrocellulose, polymethacrylate, polyvinylchloride, styrene-divinylbenzene, or any chemically-modified plastic or any other nonreacting structure. In still further embodiments, the nonreacting structure is biotin or iminobiotin. Biotin or

iminobiotin binds to avidin or streptavidin, which can be used to isolate the capture nucleic acid and any hybridizing molecules. Furthermore, in some embodiments of the invention, the nonreacting structure is cellulose or an analog thereof.

It is contemplated that the location of the targeting and bridging regions in the bridging nucleic acid may be at a variety of positions. The location of targeted regions in a targeted nucleic acid or a capture region in a capture nucleic acid may also vary. The location of any of these regions or nonreacting structure may be or be within 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 12, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900. 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000 or more nucleotides from the 3' and/or 5' end of the relevant nucleic acid ("relevant nucleic acid" refers to the nucleic acid in which the region is located). Moreover, it is contemplated that a region, such as a bridging, capture, targeted, or targeting region-as well as a nonreacting structure-may be at or within 100-5000 residues. 150-4000 residues, 200-3000 residues, 250-2000 residues, 300-1500 residues, 350-1000 residues, 400-900 residues, 450-800 residues, or 500-700 residues of the 5' or 3' end of the relevant nucleic acid.

15

20

25

30

Furthermore, it is also contemplated that the spacing between regions may vary. Regions in the same nucleic acid or a region and a nonreacting structure may be adjacent to one another or there may be residues between them or between each of them. The number of intervening residues may be the following or may be at least or at most of the following: 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 65, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590,

600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, or more nucleotides between them or each of them.

As for the location of the sequence to which the targeting region is complementary, termed "targeted region," this may be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 10 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000 nucleotides or more from the 3' and/or 5' end of the targeted nucleic acid. It is specifically contemplated that the targeting region hybridizes to a sequence located between 100 and 5000, 150 and 4000, 200 and 3000, 250 and 2000, and 300 and 1000 residues of the 5' and/or 3' end of the targeted nucleic acid. It is also contemplated that the targeted region is at the 3' or 5' end of the targeted nucleic acid. Alternatively, the targeted region may not be within 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700, 750, 800, 850, 900, 950, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000 or more nucleotides from the termini of a targeted nucleic acid.

20

25

30

In some embodiments, the targeting region comprises or is complementary to all or 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940,

950, 960, 970, 980, 990, 1000 or more contiguous nucleotides of SEQ ID NO:1, SEQ ID NO:2, SEO ID NO:3, SEO ID NO:4, SEO ID NO:5, SEO ID NO:6, SEO ID NO:7, SEO ID NO:8, SEO ID NO:9, SEO ID NO:10, SEO ID NO:11, SEO ID NO:12, SEO ID NO:13, SEO ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEO ID NO:21, SEO ID NO:22, SEO ID NO:23, SEO ID NO:24, SEO ID NO:25, SEO ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEO ID NO:32, SEO ID NO:33, SEO ID NO:34, SEO ID NO:35, SEO ID NO:36, SEO ID NO:37, SEO ID NO:38, SEO ID NO:39, SEO ID NO:40, SEO ID NO:41, SEO ID NO:42, SEO ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48, SEO ID NO:49, SEO ID NO:50, SEO ID NO:51, SEO ID NO:52, SEO ID NO:53, SEO ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:60, SEO ID NO:61, SEO ID NO:62, SEO ID NO:63, SEO ID NO:64, SEO ID NO:65. SEO ID NO:66, SEO ID NO:67, SEO ID NO:68, SEO ID NO:69, SEO ID NO:71, SEO ID NO:72, or SEO ID NO:73 (collectively referred to as "SEO ID NOS:1-73"), as well as SEO ID NO:74, SEO ID NO:75, SEO ID NO:76, SEO ID NO:77, SEO ID NO:78, SEO ID NO:79, SEO ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, SEO ID NO:86, SEO ID NO:87, SEO ID NO:88, SEO ID NO:89, SEO ID NO:91, SEO ID NO:91, and SEQ ID NO:92 (collectively referred to as "SEQ ID NOS:1-92"). It is specifically contemplated that targeting regions of the invention comprise, in some embodiments, at least 5 contiguous nucleotides of SEQ ID NO:1-22; it is also contemplated that targeting regions of the invention are complementary to a sequence ("sequence" in the context of complementary regions refers to a sequence of at least 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, or more nucleotides in length) of SEQ ID NOS:23-86, which are sequences of rRNA molecules.

It will be understood that any embodiment discussed with respect to nucleotides applies also when nucleotide analogs are used. It is specifically contemplated that nucleotide analogs may be employed with respect to bridging and capture nucleic acids of the invention.

20

25

30

It is contemplated that nucleic acids of the invention include RNA, DNA, locked nucleic acid™ (LNA), iso-bases, and/or peptide mimetics. It is contemplated that all or part of nucleic acids of the invention may include such nucleic acid components.

The present invention further concerns methods of isolating and/or depleting nucleic acids from a sample. In some embodiments, methods include a) incubating a sample with a first

bridging nucleic acid comprising (1) at least one bridging region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the first targeting region and the targeted nucleic acid; b) incubating the first bridging nucleic acid with a capture nucleic acid comprising a nonreacting structure and a capture region comprising at least 5 nucleic acid residues, under conditions that allowing hybridization between the first bridging region and the capture region. In additional embodiments, one or more other steps may be included in combination with the method discussed above. Other steps involve isolating the targeted nucleic acid from the remainder of the sample; discarding the portion of the sample that hybridizes directly or indirectly to the capture nucleic acid (indirect hybridization refers to specific association of compounds that occurs through hybridization with a mediating compound, for example, indirect hybridization of a capture nucleic acid and a targeted nucleic acid via hybridization to a bridging nucleic acid); incubating the sample with additional bridging nucleic acids, under conditions allowing hybridization between the targeting region of the additional bridging nucleic acid and the targeted nucleic acid; implementing the method with respect to other targeted nucleic acids; washing the capture nucleic acid after incubation with the sample and the bridging nucleic acid; incubating the capture nucleic acid, bridging nucleic acid, and sample with elution buffer after isolating the targeted nucleic acid from the rest of the sample; eluting the targeted nucleic acid from the nonreactant structure; using the capture nucleic acid in a subsequent method involving a new sample; discarding the targeted nucleic acid after separating it from the sample; performing hybridizations between the bridging nucleic acid and the sample and the capture nucleic acid and the sample at the same temperatures or at different temperatures; performing the above hybridization steps at the same time, sequentially (one after the other or the other after the one); exposing the sample to a magnetic field or magnet. particularly when a magnetic bead or other object comprises all or part of the non-reacting structure of the capture nucleic acid; and incubating the sample with streptavidin or avidin. particularly if biotin or iminobiotin is used as a non-reacting structure.

15

20

25

30

In some embodiments of the invention, the sample, a bridging nucleic acid and/or a capture nucleic acid are incubated in a buffer, which, in some embodiments, includes TEAC or TMAC.

In methods of the invention involving more than one bridging nucleic acid, it is contemplated that the targeting region of the first bridging nucleic acid may be complementary to

a different sequence of a different targeted nucleic acid than a targeting region of another bridging nucleic acid. Alternatively, different bridging nucleic acids may have targeting regions that are complementary to the same targeted nucleic acid. In the latter case, it is further contemplated that the targeting regions be complementary to sequences that overlap one another

or ma be complementary to sequences in non-overlapping locations.

5

10

15

20

25

30

In cases in which targeting regions are complementary to different targeted nucleic acids, embodiments may involve targeting the largest rRNA molecule in a sample with one bridging nucleic acid and the second largest rRNA molecule in a sample with another bridging nucleic acid. In still further embodiments, another or third bridging nucleic acid will target the third largest rRNA molecule in a sample, while another or a fourth bridging nucleic acid will target the fourth largest rRNA molecule in a sample.

In another embodiment of the invention, there is a method for depleting rRNA from a sample comprising incubating the sample with (1) at least a first bridging oligonucleotide comprising a bridging region comprising a polypurine region of at least 5 residues in length and a targeting region comprising at least 5 contiguous residues complementary to an rRNA molecule in the sample and (2) a capture oligonucleotide comprising a magnetic bead and a capture region comprising a polypyrimidine region of at least 5 residues in length, under conditions allowing hybridization between the bridging oligonucleotide and the capture oligonucleotide and between the bridging oligonucleotide and the rRNA; b) incubating the sample with a magnetic bead; and c) isolating the magnetic bead. In still further embodiments, the first bridging oligonucleotide comprises a targeting region complementary to prokaryotic 23S rRNA. In still further embodiments, there is a second bridging oligonucleotide with a targeting region complementary to a different region of a prokaryotic 23S RNA than the first bridging oligonucleotide. In even further embodiments, there is a third and fourth bridging oligonucleotide each with a targeting region complementary to different sequences of a prokaryotic 16S rRNA.

As discussed earlier, a sample may be depleted or isolated as a way of enriching for the nontargeted nucleic acid, such as mRNA. In further embodiments of the invention, enriched mRNA can be used to prepare cDNA according to methods known to those of ordinary skill in the art, and as described herein. Thus, in cases in which mRNA is enriched as a result of methods of the invention, embodiments may further include discarding the portion of the sample

15

20

25

30

that hybridizes to the capture oligonucleotide. More specifically targeted rRNA may be discarded and the mRNA remaining in the sample may be used to produce cDNA molecules. cDNA molecules may be used in a variety of methods, including, but not limited to, library production, production of proteins, and for creating and screening arrays. Therefore, in some embodiments of the invention, cDNA made from mRNA enriched according to methods of the invention are attached to a solid support or surface so as to create a nucleic acid array. The term "nucleic acid array" refers to a plurality of target elements, wherein each target element comprising one or more nucleic acid molecules immobilized on one or more solid surfaces at discrete locations to which sample nucleic acids can by hybridized. The nonreacting solid surface or support may be any of a number of materials, including plastic, glass, or nylon. In some embodiments, the solid support is a plate. The plate may have wells that contain the target elements. Plates may have 2, 3, 4, 5, 6, 7, 8, 9, 10 or more wells ("multi-well"), and up to at least 96 or 192 wells. In some embodiments of the invention, the sample nucleic acids comprise cDNAs made by depleting a sample of rRNA, according to methods of the invention. Those embodiments may further involve contacting a nucleic acid array with the cDNA. Alternatively, cDNA made according to the invention may be used as target elements on an array. In any of these embodiments of the invention, it is specifically contemplated that enriched mRNA may be amplified into RNA or DNA by techniques known to those of skill in the art and then used in methods of the invention, such as to probe or screen an array.

The present invention also concerns kits that include compositions of the invention to implement the methods discussed herein. These kits can be used for the depletion, isolation, or purification of nucleic acids. Kits contain these compositions in a suitable container means.

In some embodiments, a kit includes 1) at least one capture oligonucleotide comprising a capture region and a magnetic bead; and 2) at least a first bridging oligonucleotide comprising i) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and ii) at least one targeting region comprising 10 contiguous nucleic acids complementary to an rRNA.

In additional embodiments, there is a second bridging oligonucleotide comprising i) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and ii) at least one targeting region comprising 10 contiguous nucleic acids complementary to an rRNA. In some kits, the targeting region of the second bridging

oligonucleotide is complementary to the same rRNA as the targeting region of the first bridging oligonucleotide, while in other embodiments, these are complementary to different rRNAs. Further embodiments involve kits in which the targeting region of the first bridging oligonucleotide is complementary to the largest rRNA of a prokaryote or eukaryote. In other embodiments, the second bridging oligonucleotide has a targeting region that is complementary to either the largest rRNA of a prokaryote or eukaryote or the second largest rRNA of a prokaryote or eukaryote or eukaryote or enterpolated that kits may include one or more bridging oligonucleotides targeting prokaryotic rRNA (16S, 23S, or both) and one or more bridging oligonucleotides targeting eukaryotic rRNA (18S, 28S, or both); thus, a kit may be used for depleting both eukaryotic and prokaryotic rRNA in some embodiments.

5

10

15

20

25

Kits may also include a third, fourth, fifth, sixth, seventh, eighth, ninth, tenth or more bridging oligonucleotides with targeting region complementary to the same or different rRNAs as the targeting regions of the first and second bridging oligonucleotides. It is contemplated that the targeting regions of the bridging oligonucleotides in kits of the invention may be complementary to prokaryote 16S rRNA, prokaryote 23S rRNA, prokaryote 5S rRNA, eukaryote 17S or 18S rRNA, eukaryote 28SrRNA, and/or eukaryote 5.8S rRNA. It is further contemplated that targeting regions of bridging oligonucleotides in kits may have all or part of SEO ID NO:1. SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEO ID NO:9, SEO ID NO:10, SEO ID NO:11, SEO ID NO:12, SEO ID NO:13, SEO ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19. SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:22 (collectively referred to as "SEQ ID NOS:1-22"). Alternatively, kits may include targeting regions as discussed above with respect to SEO ID NOS:23-86, i.e. targeting regions complementary to a sequence from SEQ ID NOS:23-86. Kits of the invention may also include one or more of the following; binding buffer with TMAC. binding buffer with TEAC, magnetic stand, wash solution, nuclease-free water; RNAse inhibitors, glycogen, control RNA, sodium acetate, ammonium acetate, streptavidin beads. avidin beads, magnetic beads, beads of any nonreacting structure--including those discussed above--capture basket; capture filters, RNA markers, nuclease-free containers such as tubes and tips, and any other composition described herein.

It is contemplated that kits of the invention may be used to implement methods of the invention, that methods of the invention may be implemented with compositions of the invention, and that kits may include any composition of the invention.

It is further contemplated that kits, methods, and compositions of the invention may effect a depletion of a targeted nucleic acid in a sample by reducing its amount in the sample by at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 96, 97, 98, 99, 99.1, 99.2, 99.3, 99.4, 99.5, 99.6, 99.7, 99.8, 99.9 or more percent.

Kits of the invention also include materials for creating a nucleic acid array and probing a nucleic acid array. Any of the kits discussed above may also include a solid support for preparing a nucleic acid array.

10

15

20

25

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." When the term "at least" is used in the context of bridging, targeting, or capture regions, as well as for capture and bridging oligonucleotides, it is contemplated that there is an upper limit of 20 for practical purposes, even though more such regions or oligonucleotides could be implemented with the invention. Furthermore, it should be understood that a number (cardinal or ordinal) used in the context of compositions of the invention refers to a "kind" of that composition; thus, "a first oligonucleotide" in the context of a "second oligonucleotide" refers to "one of that kind of oligonucleotide," and not one single oligonucleotide molecule.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

15

20

25

30

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Depiction of molecules in system. A bridging oligonucleotide is shown with a targeting region and a bridging region. The targeting region is complementary to a targeted region in the targeted nucleic acid, which is an rRNA molecule. The bridging region is complementary to the capture region in the capture oligonucleotide, which is attached, by way of example, to a magnetic bead as a nonreacting structure.

FIG. 2A-1 to A-14 and FIG. 2B-1 to B-27. Sequence comparison of different rRNAs from different bacteria to E. coli rRNA with MegAlign sequence analysis software version 4.05 from DNA Star, Incorporated. A. The 5' end of the sequence is shown on the first page of the figure in FIG. 2A-1 and continues until the last page of the figure, FIG. 2A-14, in which the 3' end of the same sequence is shown. Shown is a sequence comparison of 16S rRNA of listed prokaryotic organisms to 16S rRNA from E. coli (SEO ID NO:34). The sequences are the 16S rRNA from the following organisms: B. subtilis (SEO ID NO:23); B. anthracis (SEO ID NO. 24); E. faecalis (SEO ID NO. 25); L. lactis (SEO ID NO. 26); L. monocyt (SEO ID NO. 27); S. aureus (SEO ID NO. 28); S. mutans (SEO ID NO. 29); S. pneumon (SEO ID NO. 30); S. pyogenes (SEQ ID NO. 31); M. avian (SEQ ID NO. 32); M. tuberculosis (SEQ ID NO. 33); K. pneumoniae (SEO ID NO. 35); A. actino (SEO ID NO. 36); H. influenzae (SEO ID NO. 37); E. bronchiseptica (SEQ ID NO. 38); B. parapertussis (SEQ ID NO. 39); B. pertussis (SEQ ID NO. 40); B. cepacia (SEO ID NO. 41); B. mallei (SEO ID NO. 42); B. pseudomallei (SEO ID NO. 43); N. gonorrhoeae (SEO ID NO. 44); N. mening (SEO ID NO. 45); P. aeruginosa (SEO ID NO. 46); V. cholerae (SEQ ID NO. 47); and Y. enterocolitica (SEQ ID NO. 48). B. The 5' end of the sequence is shown on the first page of the figure in FIG. 2B-1 and continues until the last page of the figure, FIG. 2B-27, in which the 3' end of the same sequence is shown. Shown is a sequence comparison of 23S rRNA of listed prokaryotic organisms to 23S rRNA from E. coli (SEQ ID NO:60). The sequences are the 23S rRNA from the following organisms: B. subtilis (SEQ ID NO:49); B. anthracis (SEQ ID NO. 50); E. facaelis (SEQ ID NO. 51); L. lactis (SEQ ID

WO 03/054162 PCT/US02/41014

17

- NO. 52); L. monocytogenes (SEQ ID NO. 53); S. aureus (SEQ ID NO. 54); S. mutans (SEQ ID NO. 55); S. pneumoniae (SEQ ID NO. 56); S. pyogenes (SEQ ID NO. 57); M. avium (SEQ ID NO. 58); M. tuberculosis (SEQ ID NO. 59); K. pneumoniae (SEQ ID NO. 61); H. influenzae (SEQ ID NO. 62); B. bronchiseptica (SEQ ID NO. 63); B. parapertussis (SEQ ID NO. 64); B. pertussis (SEQ ID NO. 65); B. cepacia (SEQ ID NO. 66); E. mallei (SEQ ID NO. 67); E. pseudomallei (SEQ ID NO. 68); N. gonorrhoeae (SEQ ID NO. 69); N. emingititidis (SEQ ID NO. 70); P. aeruginosa (SEQ ID NO. 71); V. cholerae (SEQ ID NO. 72); Y. enterocolitica (SEQ ID NO. 73).
- FIG. 3. Electropherograms of RNA from a control reaction. E. coli total RNA

 10 was purified with RNAwiz[™] (Ambion) and carried through the rRNA depletion procedure as described in Example 2, except that bridging nucleic acids were left out of the reaction. A sample of the RNA was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
 - FIG. 4. Electropherograms of RNA from an experimental reaction after ribosomal RNA depletion. E. coli total RNA was purified with RNAwiz™ (Ambion) and carried through the rRNA depletion procedure as described in Example 2. A sample of the RNA was analyzed as described in the legend to FIG. 3.

15

FIG. 5A-B. Electropherograms of RNA from experiments. A. Agilent 2100

Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 5, but with no bridging oligonucleotides. The sample contains E. coli and rat liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 5 with bridging oligonucleotides. The sample is depleted of E. coli 16S and 23S rRNA and rat liver 18S and 28S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 30 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

FIG. 6A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 6, but with no bridging oligonucleotides. The sample contains human liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer of a sample from an experimental reaction performed as described in Example 6 with bridging oligonucleotides. The sample is depleted of human 18S and 28S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

10

15

- FIG. 7A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 7, but with no bridging oligonucleotides. The sample contains rat liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 6 with bridging oligonucleotides. The sample is depleted of rat 18S and 28S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer (Bio Sizing software (Version A.02.01).
- FIG. 8A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 6, but with no bridging oligonucleotides. The sample contains mouse liver total RNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 8 with bridging oligonucleotides. The sample is depleted of mouse 18S and 28S

rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

- 5 FIG. 9A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Example 11 with no bridging oligonucleotides. The sample contains human total RNA (50 μg) and E. coli total RNA (500 ng). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The 10 electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 11 with bridging oligonucleotides. The sample is depleted of human 18S and 28S rRNA, but E. coli total RNA remains in the sample. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
- FIG. 10A-C. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer clectropherogram of a sample from a control reaction with no bridging oligonucleotides performed as described in Example 12. The sample contains rat liver total RNA (25 μg) and E. coli total RNA (2 μg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software 25 (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 12 with bridging oligonucleotides. The sample is depleted of rat 18S and 28S rRNA, but E. coli total RNA remains in the sample. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). C. Agilent

30

2100 Bioanalyzer electropherogram of a sample from an experimental reaction performed as described in Example 12 with bridging oligonucleotides. The sample is depleted of rat 18S and 28S rRNA and *E. coli* 16S and 23S rRNA. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

FIG. 11A-B. E. coli gene arrays probed with cDNA from experiments. Experimental reactions were performed as described in Example 13. RNA samples were used to generate radiolabeled cDNA for use as probes with replicate portions of Sigma-Genosys PanoramaTM E. coli gene arrays. A. E. coli gene array probed with a sample from a control reaction. The sample contains human total RNA (25 μg) and E. coli total RNA (2 μg). B. E. coli gene array probed with an RNA sample that was depleted of human 18S and 28S rRNA and E. coli 16S and 23S rRNA.

- FIG. 12A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer 15 electropherogram of a sample from a control reaction performed as described in Examples 1 and 2 with no bridging oligonucleotides. The sample contains Campylobacter fetus total RNA (10 ug). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit® (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. 20 Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction with 10 ug of Campylobacter fetus total RNA that employed the bridging oligonucleotides d16S-807. d16S-1092, d23S-479CH, and d23S-2511. The sample is depleted of 16S rRNA, 23S rRNA fragment (1260 nt), and 23S rRNA fragment (1667 nt). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer 25 (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).
 - FIG. 13A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Examples 1 and 2 with no bridging oligonucleotides. The sample contains *Rhodobacter sphaeroides* total RNA (10 μg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper

Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction with 10 μg of *Rhodobacter sphaeroides* total RNA that employed the bridging oligonucleotides d16S-357 (16 pmol), d16S-1114R(16 pmol), d23S-479CH (16 pmol), d23S-1954 (16 pmol), and d23S-2511 (16 pmol). The sample is depleted of the 16S rRNA and the 23S rRNA fragment that co-migrates with the 16S rRNA. The sample is also depleted of the 23S rRNA fragment (1260 nt). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

FIG. 14A-B. Electropherograms of RNA from experiments. A. Agilent 2100 Bioanalyzer electropherogram of a sample from a control reaction performed as described in Examples 1 and 2 with no bridging oligonucleotides. The sample contains Anabaena sp. total RNA (10 µg). The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01). B. Agilent 2100 Bioanalyzer electropherogram of a sample from an experimental reaction with 10 µg of Anabaena sp. total RNA that employed the bridging oligonucleotides d16S-364 (12.5 pmol), d16S-1087CY (12.5 pmol), d23S-485 (20 pmol), and d23S-1954 (35 pmol). The sample is depleted of 16S rRNA and the 23S rRNA fragments at 520 nt, 2090 nt, and 2470 nt. The RNA sample was analyzed with the RNA 6000 Lab Chip Kit[®] (Caliper Technologies Corp.) using the Agilent 2100 Bioanalyzer (Agilent Technologies). The electropherogram shown was generated with Agilent 2100 Bioanalyzer Bio Sizing software (Version A.02.01).

25

30

10

15

20

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention concerns a system for isolating, depleting, or identifying specific, targeted nucleic acid populations, such as rRNA in a sample, in some cases for the purpose of enriching for other nucleic acid populations. The targeted nucleic acid, components of the system, and the methods for implementing the system, as well as variations thereof, are provided below.

10

15

20

25

30

I. Targeted Nucleic Acid

The present invention concerns targeting a particular nucleic acid population (i.e., mRNA, rRNA, tRNA, genomic DNA) or targeting types of a nucleic acid population, such as individual tRNAs, rRNAs (5S, 16S, or 23S rRNA from prokaryotes; 5.8S, 17S or 18S, or 28S from eukaryotes), or specific mRNAs. A nucleic acid is targeted by using a bridging nucleic acid that has a targeting region—a region complementary to all or part of the targeted nucleic acid.

In some embodiments, the invention is specifically concerned with depleting or isolating rRNA from other nucleic acids ("non-targeted nucleic acids" or "enriched population"). The 5S, 16S, and/or 23S rRNA from a prokarvote may be the targeted nucleic acid. Also, the 5.8S, 17S (observed in yeast) or 18S, and/or 28S from a eukaryote may be the targeted nucleic acid. Alternatively, rRNAs from both prokaryotes and eukaryotes may be targeted, such as with a sample that has eukaryotic host cells infected with a prokaryotic organism. The sequences for ribosomal RNAs are well known to those or ordinary skill in the art and can be readily found in sequence databases such as GenBank (www.ncbi.nlm.nih.gov/) or are published. Nucleic acids may be targeted by targeting regions that are complementary to all or part of the targeted nucleic acid. Targeted nucleic acids may be, be at least, or be at most 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800, 810, 820, 830, 840, 850, 860, 870, 880, 890, 900, 910, 920, 930, 940, 950, 960, 970, 980, 990, 1000, 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, 1900, 2000, 2100, 2200, 2300, 2400, 2500, 2600, 2700, 2800, 2900, 3000, 3100, 3200, 3300, 3400, 3500, 3600, 3700, 3800, 3900, 4000, 4100, 4200, 4300, 4400, 4500, 4600, 4700, 4800, 4900, 5000, 5100, 5200, 5300, 5400, 5500, 5600, 5700, 5800, 5900, 6000, 6100, 6200, 6300, 6400, 6500, 6600, 6700, 6800, 6900, 7000, 7100, 7200, 7300, 7400, 7500, 7600, 7700, 7800, 7900, 8000, 8100, 8200, 8300, 8400, 8500, 8600, 8700, 8800, 8900, 9000, 9100, 9200, 9300, 9400, 9500, 9600, 9700, 9800, 9900, 10000, or more nucleotides in length. Furthermore, any region of at least five contiguous nucleotides in the targeted nucleic acid may be used as the targeted region—that is, the region that is complementary to the targeting region of a bridging nucleic acid. Also, there may be more than one targeted region in a targeted nucleic acid. There may be, be at least, or be at most 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more targeted regions in a targeted nucleic acid. A targeted region may be a region in a targeted nucleic acid that has greater than 70%, 80%, or 90% homology with a sequence from a different targeted nucleic acid. In some embodiments, the targeted region from a targeted nucleic acid is identical to a sequence in a different targeted nucleic acid. For example, 23S rRNA of various prokaryotes may be targeted using a targeted region common to a group of organisms, such as Gram negative bacteria or a subset of such bacteria. Alternatively, a targeted region may be a sequence unique to a particular targeted nucleic acid. However, for purposes of this application, a "targeted region" is not a poly-A region, such as a poly-A tail of an eukaryotic mRNA. Additional information regarding targeted rRNAs is provided below. This information is provided as an example of targeted nucleic acids. However, it is contemplated that there may be sequence variations from individual organism to organism and these sequences provided as simply an example of one sequenced nucleic acid, even though such variations exist in nature. It is contemplated that these variations may also be targeted, and this may or may not require changes to a targeting nucleic acid or to the hybridization conditions, depending on the variation, which one of ordinary skill in the art could evaluate and determine.

A number of patents concern a targeted nucleic acid, for example, U.S. Patent Nos. 4,486,539; 4,563,419; 4,751,177; 4,868,105; 5,200,314; 5,273,882; 5,288,609; 5,457,025; 5,500,356; 5,589,335; 5,702,896; 5,714,324; 5,723,597; 5,759,777; 5,897,783; 6,013,440; 6,060,246; 6,090,548; 6,110,678; 6,203,978; 6,221,581; 6,228,580; and WO 01/32672, all of which are specifically incorporated herein by reference.

A. Prokaryotic rRNA

15

20

25

30

Prokaryotic rRNA can be a targeted nucleic acid of the invention. The following examples are provided, but the invention is not limited solely to these organisms and sequences (GenBank accession number provided and/or region within sequence that corresponds to the targeted rRNA):

Superkingdom Archaea (archaebacteria)

		s jannaschii NC_00090	9		
	MJrm.		157985-159459		
	MJrm.		154759-157648		
5	Halobacterium	n marismortui	3710000		
3	23S Halohaatanium	sp. NRC-1 NC 002607	X13738		
	тз (16		1875505-1876977		
	πlA (2		1877506-1880411		
	Thermoplasma		1077500 1000711		
10	23S	•	M32298		
		acidophilum NC_0025			
_	16S		1475300-1475770		
2.	Superkingdo	m <u>Eubacteria</u> (eubac	teria)		
	a. Firmi	cutes (Gram-positive	bacteria)		
15	i)		n group (low G+C gram-positive		
		bacteria) Listeria innocua Clip 1	1262 NC 002212		
		16S	260527-262081		
		23S	262327-265257		
20					
			Listeria monocytogenes strain EGD NC_003210		
		16S	237466-239020		
		23S	239265-242195		
25		Bacillus subtilis NC00	0964		
		RmO 16S	9809-11361		
•		RmA 23S	11707-14634		
		Bacillus anthracis			
30		16S (1508nt)	AF155950		
		23S (2922nt)	AF267877		
		Bacillus thuringiensis			
		16S (1486nt)	D16281		
35		23S (2923nt)	AF267880		
		0. 1.1			
		Staphylococcus aureus 16S	strain Mu50 NC_002758 530479-532033		
		23S	532398-535231		
40		233	332370-333231		
		Staphylococcus aureus	N315 NC 002745		
		SarRNA01 16S	506138-507692		
		SarRNA02 23S	508166-510999		
45		Clostridium acetobutvli	cum ATCC824 NC 003030		
•		16SarRNA	9710-11219		
		23SarRNA	11398-14303		
50		Clostridium difficile	3/72460		
.50		16S (1470nt)	X73450		

		Clostridium perfringens	
		168	M69264 (499-2294)
		Mycoplasma genitalium G3	7 L43967
5		MgrmA16S	170009-171527
		MgrmA23S	171730-174463
		Mycoplasma pneumoniae N	VC_000912
		16S	118312-119824
10		23S	120057-122961
		Mycoplasma pulmonis NC	_002771
		16S	813583-815113
15		23\$	810563-813297
13		Streptococcus pneumoniae	R6 NC_003098
		RRNA16S-1	15161-16674
		RRNA23S-1	16945-19846
20		Streptococcus pneumoniae	ΓΙGR4 AE005672
		SprmaA16S	15394-16806
		SprmaA23S	17142-20043
		Streptococcus pyogenes AE	004092
25		16S	17170-18504
		23S	19037-21937
		Streptococcus mutans	
		16S (1334nt)	X58303
30		238	AF139599 (1940-4840)
		Lactococcus lactis	
		16S	X64887 (508-2055)
35		23\$	X64887 (2360-5257)
33		Enterococcus faecalis	
		16S (1449nt)	Y18293
		23S (2912nt)	AJ295306
40	ii)	Actinobacteria (high G+	C gram-positive bacteria
	-	Mycobacterium leprae strain	n TN NC_002677
		Rrs16S	1341144-1342692
		Rrl23S	1342976-1346100
45		Mycobacterium tuberculosis	
		MtrmaA16S	1471388-1472923
		MtrmaA23S	1473199-1476336
		Mycobacterium avium	
50		16S (1372nt)	M61673
		238	X74494 (295-3401)

			Corynebacterium glut 16S (1479nt)	amicum Z46753
5			Rhodococcus equi 16S (1478nt)	X80614
	b.	Spire	ochaetales (spirochete	s)
		Rorre	elia burgdorferi AE0007	83
			RrlB 16S	444581-446118
10			RrlB 23S	438590-441508
		Trepo	onema pallidum AE0005	
			TprmaA16S	230162-231656
16			TprmaA23S	231950-234850
15		Rorra	lia burgdorferi	
		20110	16S	AE001147 (9459-10996)
			238	AE001147 (212-3145)
20	c.	Ther	motogales	
		Thern	notoga maritima AE000.	512
			TmrmaA16S	188968-190526
			TmrmaA23S	190766-193787
25	d.	Ther	mus/Deinococcus gro	up
		Deino	coccus radiodurans R1	NC_001263
			DrrmaA16S	2285518-2287019
			DrrmaA23S	2245319-2246194
30		Deino	ococcus radiodurans	
			16S	AE002076 (7275-8776)
			23S	AE001886 (8829-10771)
	e.	Chla	mydiales (chlamydias)
35		Chlan	nydia trachomatis AE00	
			16SrRNA1	854128-855677
			23SrRNA1	855993-858862
		Chlan	nydophila pneumoniae A	R39 NC_002179
40			CprmA16S	1069329-1070785
			CprmA23S	1066159-1069022
		Chlan	nydophila psittaci	
			16S	U68447 (1-1553)
45			23S	U68447 (1778-4721)
	f.	Prote	eobacteria (purple bac	cteria)
		i)	Alpha subdivision Rickettsia conorii Mali	ish 7 NC_003103

		27		
		Rrs16S Rr123S	884601-886108 281797-284557	
		•		
_		Rickettsia prowazekii strain Madrid E AJ235269		
5			772263-773769	
		Rri23S	257853-260613	
		Rickettsia typhi		
		16S (1444nt)	M20499	
10		23S	Y13133 (956-3716)	
		Ehrlichia bovis		
		16S (1488nt)	U03775	
15		Agrobacterium tumefaciens C58	AF007870	
13			768991-770427	
		238	765313-767565	
		Brucella melitensis		
20		168	AF220148 (645-2129)	
		23S AF220148	(2896-30243204-5807)	
		Rhizobium rhizogenes		
		16S (1369nt)	D13945	
25				
	ii)	Beta subdivision		
		Neisseria meningitides strain M		
		NmrmaA16S 60971-6		
30		NmrmaA23S 63178-6	56068	
30		Bordetella bronchiseptica		
		168 (1532nt)	X57026	
		23S (2865nt)	X70371	
35		Bordetella parapertussis	770 10 10	
		16S (1464nt) 23S (2865nt)	U04949 X68368	
		23S (2863fit)	A08306	
		Bordetella pertussis		
40		16S (1464nt)	U04950	
		Burkholderi mallei		
		16S (1488nt)	AF110188	
		23S (2882nt)	Y17183	
45		255 (200211)		
		Burkholderi pseudomallei		
		16S (1488nt)	U91839	
		23S (2882nt)	Y17184	
50		Neisseria gonorrhoeae		
30			X07714	
			X67293	
		200 (200 0110)		

	iii)	Gamma group		
		Buchnera sp. APS NC_00252	28	
		Rrs 16S	274065-275524	
5		Rrl 23S	539539-542451	
		Escherichia coli K12 U00096		
		RrsH 16S	223771-225312	
10		RrlH 23S	225759-228662	
10		Escherichia coli 0157:H7 NC	_002695	
		RrsH 16S	227102-228643	
		RrlH 23S	229090-231992	
15		Salmonella enterica serovar T	yphi NC 003198	
		168	287479-289020	
		23S	289375-292380	
		Salmonella typhimurium LT2	NC 003197	
20		RrsH16S	289189-290732	
		RrlH23S	291244-294336	
		Yersinia pestis NC_003143		
		i6S -	12292-13763	
25		23\$	14272-17178	
		Klebsiella pneumoniae		
		16S (1534nt)	X87276	
20		23S (2903nt)	X87284	
30		Yersinia enterocolitica		
		16S (1484nt)	Z49830	
		23S (2906nt)	U77925	
35		Proteus vulgaris		
		16S (2067nt)	X07652	
		Shigella flexneri		
40		16S (1468nt)	X80679	
40		Shigella sonnei		
		16S (1467nt)	X80726	
		Shigella dysenterica		
45		16S (1487nt)	X96966	
		Haemophilus influenzae Rd L42023		
		HirmE16S	1511137-1512634	
50		HirmE23S	123801-126697	
50		Pasteurella multocida		
		16S (1543nt)	M35018	

		Actinobacillus actinomycetemcomitans	
		16S (1485nt)	M75037
5		Actinobacillus pleuropneumon	iae
•		16S	D30032 (83-1625)
		Haemophilus somnus	
10		16S (1483nt)	M75046
10		Legionella pneumophila	
		16S (1544nt)	M59157
		Mannheimia haemolytica	
15		16S (1472nt)	U57072
		Vibrio cholerae chromosomel	
		16Sa rRNA	53823-55357
20		23Sa rRNA	55784-58670
		Vibrio parahaemolyticus	
		16S (1499nt)	M59161
		Coxiella burnetii	
25		16S (1484nt)	M21291
		23S	X79704 (1620-3350)
		Aeromonas hydrophila	
30		16S (1538nt)	X87271
50		Aeromonas salmonicida	
		16S (1502nt)	X60405
		Francisella tularesis	
35		16S (1517nt)	Z21931
		Moraxella catarrhalis	
		16S (1511nt)	U10876
40		Pseudomonas aeruginosa AE0	
		16S	722096-726631
		23S	724103-726993
		Pseudomonas putida	
45		16S (1527nt)	D84020
	iv)	Delta/Epsilon subdivisions	
		Campylobacter jejuni AL1111	68
50		168	39249-40761
		23\$	41568-44457

Helicobacter pylori 26695 NC_000915 HPrmB16S 1511137-1512634 HPrmB23S 1473918-1476893

g. Cyanobacteria

5

10

15

20

Synechocystis sp. PCC 6803 NC_000911

Rm16Sa 2452187-2453675 Rm23Sa 2448839-2451721

Synechococcus sp. (Anacystis nidulans) 16S X03538 (1432-2918) 23S X00512 (251-3126)

h. CFB/Green sulfer bacteria group

Porphyromonas gingivalis 16S (1474nt) L16492

B. Eukaryotic rRNA

Targeted nucleic acids of the invention may also be one or more types of eukaryotic rRNAs. Eukaryotes include, but are not limited to: mammals, fish, birds, amphibians, fungi, and plants. The following provides sequences for some of these targeted nucleic acids. It is contemplated that other eukaryotic rRNA sequences can be readily obtained by one of ordinary skill in the art, and thus, the invention includes, but is not limited to, the sequences shown below.

25	Superkingdom <u>Eukaryota</u> (eucar Homo sapiens (human)	yotes)
	188	M10098
	188	K03432
	188	X03205
30	28S	M11167
	Mus muculus	
	18S	X00686
	28S	X00525
35		
	Rattus norvegicus	
	188	M11188
	188	X01117
40	Rattus norvegicus V01270.1	
	18S	1-1874
	288	3862-8647

II. Isolation and/or Depletion System Nucleic Acids

The present invention concerns compositions comprising a nucleic acid or a nucleic acid analog in a system or kit to deplete, isolate, or separate a nucleic acid population from other nucleic acid populations, for which enrichment may be desirable. It concerns a bridging nucleic acid and a capture nucleic acid to deplete, isolate, or separate out a targeted nucleic acid, as discussed above.

A. Bridging Nucleic Acids

15

20

25

30

Bridging nucleic acids of the invention comprise a bridging region and a targeting region.

As discussed in other sections, the location of these regions may be throughout the molecule, which may be of a variety of lengths. The bridging nucleic acid may comprise RNA, DNA, both, or analogs of either or both.

The bridging region comprises a sequence that is complementary to at least five contiguous nucleotides in the capture nucleic acid. It is contemplated that that this region may be a homogenous sequence, that is, have the same nucleotide repeated across its length, such as a repeat of A, C, G, T, or U residues. However, to avoid hybridizing with a poly-A tailed mRNA in a sample comprising eukaryotic nucleic acids, it is contemplated that most embodiments will not have a poly-U or poly-T bridging region when dealing with such samples having poly-A tailed RNA. In some embodiments, the bridging region is a poly-C region and the capture region is a poly-G region, or vice versa. In other embodiments, the bridging region will be a random sequence that is complementary to the capture region (or the capture region will be random and the bridging region will be complementary to it). In further embodiments, the bridging region will have a designed sequence that is not homopolymeric but that is complementary to the capture region or vice versa. Sequences may be determined empirically. In many embodiments, it is preferred that this will be a random sequence or a defined sequence that is not a homopolymer. Some sequences will be determined empirically during evaluation in the assav.

B. Capture Nucleic Acids

Capture nucleic acids of the invention comprise a capture region and a nonreacting structure that allows the capture nucleic acid, any molecules specifically binding or hybridizing to the capture nucleic acid—such as the bridging nucleic acid—and any molecules specifically binding or hybridizing to the bridging nucleic acid—such as the targeted nucleic acid—to be isolated away from other nucleic acid populations.

25

30

The capture nucleic acid may comprise RNA, DNA, both, or analogs of either or both. However, in some embodiments of the invention, it is specifically contemplated to be homopolymeric (only one type of nucleotide residue in molecule, such as poly-C), though in other embodiments, it is specifically contemplated not to be homopolymeric and be heteropolymeric, as described for bridging regions.

Capture Regions

The main requirement for bridging and capture nucleic acid sequences is that they are complementary to one another. The capture region may be a poly-pyrimidine or poly-purine region comprising at least 5 nucleic acid residues. In addition, it may be heteropolymeric, either a random sequence or a designed sequence that is complementary to the bridging region of the nucleic acid with which it should hybridize.

In addition to the capture oligos already described herein, the following are also considered for use with the present invention:

NRS-5'-TAACCTGGTCGTAAC-3' (SEQ ID NO:87)

NRS-5'-CCCCCCCCCCCCCC3' (SEQ ID NO:88)

NRS-5'-GCCCCTAACCTCGTCG (SEQ ID NO:89)

20 NRS-5'-CGGCCCTAGCCGGGTCGTACCTCCGG (SEQ ID NO:90)

NRS-5'-CGGCCCTAACCTGGTCGTAACTCCGG (SEQ ID NO:91)

NRS-5'-AGGCTTCGATCCCGGGATCCGCG (SEQ ID NO:92)

As discussed below, "NRS" refers to a non-reacting structure.

2. Nonreacting Structures (NRS)

A nonreacting structure is a compound or structure that will not react chemically with nucleic acids, and in some embodiments, with any molecule that may be in a sample. Nonreacting structures may comprise plastic, glass, teflon, silica, a magnet, a metal such as gold, carbon, cellulose, latex, polystyrene, and other synthetic polymers, nylon, cellulose, nitrocellulose, polymethacrylate, polyvinylchloride, styrene-divinylbenzene, or any chemically-

modified plastic. They may also be porous or non-porous materials. The structure may also be a particle of any shape that allows the targeted nucleic acid to be isolated, depleted, or separated. It may be a sphere, such as a bead, or a rod, or a flat-shaped structure, such as a plate with wells. Also, it is contemplated that the structure may be isolated by physical means or electromagnetic means. For example, a magnetic field may be used to attract a non-reacting structure that includes a magnet. The magnetic field may be in a stand or it may simply be placed on the side of a tube with the sample and a capture nucleic acid that is magnetized. Examples of physical ways to separate nucleic acids with their specifically hybridizing compounds are well known to those of skill in the art. A basket or other filter means may be employed to separate the capture nucleic acid and its hybridizing compounds (direct and indirect). The non-reacting structure and sample with nucleic acids of the invention may be centrifuged, filtered, dialyzed, or captured (with a magnet). When the structure is centrifuged it may be pelleted or passed through a centrifugible filter apparatus. The structure may also be filtered, including filtration using a pressure-driven system. Many such structures are available commercially and may be utilized herewith. Other examples can be found in WO 86/05815, WO90/06045, U.S. Patent 5,945,525, all of which are specifically incorporated by reference.

10

15

20

30

Cellulose is a structural polymer derived from vascular plants. Chemically, it is a linear polymer of the monosaccharide glucose, using β , 1-4 linkages. Cellulose can be provided commercially, including from the Whatman company, and can be chemically sheared or chemically modified to create preparations of a more fibrous or particulate nature. CF-1 cellulose from Whatman is an example that can be implemented in the present invention.

Synthetic plastic or glass beads may be employed in the context of the invention. The beads may be complexed with avidin or streptavidin and they may also be magnetized. The complexed streptavidin can be used to capture biotin linked to an oligo-dT or -U or poly (dT) or poly(U) moiety, either before or after hybridization to the poly(A) tails of mRNA. Alternatively, the oligo/poly(dT/U) moiety can be attached to the beads directly through chemical coupling. The beads may be collected using gravity- or pressure-based systems and/or filtration devices. If the beads are magnetized, a magnet can be used to separate the beads from the rest of the sample. The magnet may be employed with a stand or a stick or other type of physical structure to facilitate isolation.

Other components include isolation apparatuses such as filtration devices, including spin filters or spin columns.

C. Nucleic Acid Compositions

10

15

20

25

30

Embodiments of the present invention concern bridging, capture, and targeted nucleic acids. In particular aspects, a targeted nucleic acid encodes for or comprises a transcribed nucleic acid. In other aspects, a bridging nucleic acid comprises a targeting region that comprises a nucleic acid segment having the sequence of all or part of SEO ID NO:1, SEO ID NO:2. SEO ID NO:3. SEO ID NO:4. SEO ID NO:5. SEO ID NO:6. SEO ID NO:7. SEO ID NO:8. SEO ID NO:9. SEO ID NO:10. SEO ID NO:11. SEO ID NO:12. SEO ID NO:13. SEO ID NO:14, SEO ID NO:15, SEO ID NO:16, SEO ID NO:17, SEO ID NO:18, SEO ID NO:19, SEO ID NO:20, SEO ID NO:21, SEO ID NO:22, SEO ID NO:23, SEO ID NO:24, SEO ID NO:25, SEO ID NO:26, SEO ID NO:27, SEO ID NO:28, SEO ID NO:29, SEO ID NO:30, SEO ID NO:31, SEO ID NO:32, SEO ID NO:33, SEO ID NO:34, SEO ID NO:35, SEO ID NO:36, SEO ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEO ID NO:42. SEO ID NO:43, SEO ID NO:44, SEO ID NO:45, SEO ID NO:46, SEO ID NO:47, SEO ID NO:48, SEO ID NO:49, SEO ID NO:50, SEO ID NO:51, SEO ID NO:52, SEO ID NO:53, SEQ ID NO:54, SEO ID NO:55, SEO ID NO:56, SEO ID NO:57, SEO ID NO:58, SEO ID NO:59, SEO ID NO:60, SEO ID NO:61, SEO ID NO:62, SEO ID NO:63, SEO ID NO:64, SEO ID NO:65, SEO ID NO:66, SEO ID NO:67, SEO ID NO:68, SEO ID NO:69, SEO ID NO:71, SEO ID NO:72, or SEO ID NO:73 (collectively referred to as "SEO ID NOS:1-73"), as well as SEO ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEO ID NO:80, SEO ID NO:81, SEO ID NO:82, SEO ID NO:83, SEO ID NO:84, SEO ID NO:85, SEO ID NO:86, SEO ID NO:87, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:91, and SEQ ID NO:92 (collectively referred to as "SEQ ID NOS:1-92"). In particular aspects, a targeted nucleic acid encodes a protein, polypeptide, peptide. Nucleic acids of the invention comprise RNA, DNA, analogs of RNA, analogs of DNA, or a combination thereof.

The term "nucleic acid" is well known in the art. A "nucleic acid" as used herein will generally refer to a molecule (i.e., a strand) of DNA, RNA or a derivative or analog thereof, comprising a nucleobase. A nucleobase includes, for example, a naturally occurring purine or pyrimidine base found in DNA (e.g., an adenine "A," a guanine "G," a thymine "T" or a cytosine "C") or RNA (e.g., an A, a G, an uracil "U" or a C). The term "nucleic acid" encompass the

0 ...

5

10

15

20

25

30

terms "oligonucleotide" and "polynucleotide," each as a subgenus of the term "nucleic acid."

The term "oligonucleotide" refers to a molecule of between about 3 and about 100 nucleobases in length. The term "polynucleotide" refers to at least one molecule of greater than about 100 nucleobases in length.

These definitions generally refer to a single-stranded molecule, but in specific embodiments will also encompass an additional strand that is partially, substantially or fully complementary to the single-stranded molecule. Thus, a nucleic acid may encompass a double-stranded molecule or a triple-stranded molecule that comprises one or more complementary strand(s) or "complement(s)" of a particular sequence comprising a molecule. As used herein, a single stranded nucleic acid may be denoted by the prefix "ss," a double stranded nucleic acid by the prefix "ds," and a triple stranded nucleic acid by the prefix "ts."

1. Nucleobases

As used herein a "nucleobase" refers to a heterocyclic base, such as for example a naturally occurring nucleobase (i.e., an A, T, G, C or U) found in at least one naturally occurring nucleic acid (i.e., DNA and RNA), and naturally or non-naturally occurring derivative(s) and analogs of such a nucleobase. A nucleobase generally can form one or more hydrogen bonds ("anneal" or "hybridize") with at least one naturally occurring nucleobase in manner that may substitute for naturally occurring nucleobase pairing (e.g., the hydrogen bonding between A and T, G and C, and A and U).

"Purine" and/or "pyrimidine" nucleobase(a) encompass naturally occurring purine and/or pyrimidine nucleobases and also derivative(s) and analog(s) thereof, including but not limited to, those of a purine or pyrimidine substituted by one or more of an alkyl, caboxyalkyl, amino, hydroxyl, halogen (i.e., fluoro, chloro, bromo, or iodo), thiol or alkylthiol moiety. Preferred alkyl (e.g., alkyl, caboxyalkyl, etc.) moieties comprise of from about 1, about 2, about 3, about 4, about 5, to about 6 carbon atoms. Other non-limiting examples of a purine or pyrimidine include a deazapurine, a 2-6-diaminopurine, a 5-fluorouracil, a xanthine, a hypoxanthine, a 8-bromoguanine, a 8-thioguanine, a bromothymine, a 8-aminoguanine, a 8-thydroxyguanine, a 8-methylguanine, a 8-thioguanine, a 1-gentyleyosine, a 5-ethyluracil, a 5-iodouracil, a 5-chlorouracil, a 5-propyluracil, a 1-indouracil, a 2-methyladenine, a mazadenines, a 8-bromoadenine, a 8-thydroxyadenine, a 6-thiopurine, a

4-(6-aminohexyl/cytosine), and the like. A table of non-limiting, purine and pyrimidine derivatives and analogs is also provided herein below.

Table 1-Purine and Pyrimidine Derivatives or Analogs			
Abbr.	Modified base description	Abbr.	Modified base description
ac4c	4-acetylcytidine	Mam5s2u	5-methoxyaminomethyl-2- thiouridine
Chm5u	5-(carboxyhydroxylmethyl) uridine	Man q	Beta,D-mannosylqueosine
Cm	2'-O-methylcytidine	Mcm5s2u	5-methoxycarbonylmethyl-2- thiouridine
Cmnm5s2u	5-carboxymethylamino-methyl-2- thioridine	Mcm5u	5- methoxycarbonylmethyluridi ne
Cmnm5u	5- carboxymethylaminomethyluridin e	Mo5u	5-methoxyuridine
D	Dihydrouridine	Ms2i6a	2-methylthio-N6- isopentenyladenosine
Fm	2'-O-methylpseudouridine	Ms2t6a	N-((9-beta-D-ribofuranosyl- 2-methylthiopurine-6- yl)carbamoyl)threonine
Gal q	Beta,D-galactosylqueosine	Mt6a	N-((9-beta-D- ribofuranosylpurine-6-yl)N- methyl-carbamoyl)threonine
Gm	2'-O-methylguanosine	Mv	Uridine-5-oxyacetic acid methylester
I	Inosine	o5u	Uridine-5-oxyacetic acid (v)
I6a	N6-isopentenyladenosine	Osyw	Wybutoxosine
mla	1-methyladenosine	P	Pseudouridine

Table 1-Purine and Pyrimidine Derivatives or Analogs				
Abbr.	Modified base description	Abbr.	Modified base description	
mlf	1-methylpseudouridine	Q	Queosine	
mlg	1-methylguanosine	s2c	2-thiocytidine	
mlI	1-methylinosine	s2t	5-methyl-2-thiouridine	
m22g	2,2-dimethylguanosine	s2u	2-thiouridine	
m2a	2-methyladenosine	s4u	4-thiouridine	
m2g	2-methylguanosine	T	5-methyluridine	
m3c	3-methylcytidine	t6a	N-((9-beta-D- ribofuranosylpurine-6- yl)carbamoyl)threonine	
m5c	5-methylcytidine	Tm	2'-O-methyl-5-methyluridine	
m6a	N6-methyladenosine	Um	2'-O-methyluridine	
m7g	7-methylguanosine	Yw	Wybutosine	
Mam5u	5-methylaminomethyluridine	x	3-(3-amino-3- carboxypropyl)uridine, (acp3)u	

A nucleobase may be comprised of a nucleoside or nucleotide, using any chemical or natural synthesis method described herein or known to one of ordinary skill in the art.

Nucleosides

5

As used herein, a "nucleoside" refers to an individual chemical unit comprising a nucleobase covalently attached to a nucleobase linker moiety. A non-limiting example of a "nucleobase linker moiety" is a sugar comprising 5-carbon atoms (i.e., a "5-carbon sugar"), including but not limited to a deoxyribose, a ribose, an arabinose, or a derivative or an analog of a 5-carbon sugar. Non-limiting examples of a derivative or an analog of a 5-carbon sugar 10 include a 2'-fluoro-2'-deoxyribose or a carbocyclic sugar where a carbon is substituted for an oxygen atom in the sugar ring.

Different types of covalent attachment(s) of a nucleobase to a nucleobase linker mojety are known in the art. By way of non-limiting example, a nucleoside comprising a purine (i.e., A or G) or a 7-deazapurine nucleobase typically covalently attaches the 9 position of a purine or a 7-deazapurine to the 1'-position of a 5-carbon sugar. In another non-limiting example, a nucleoside comprising a pyrimidine nucleobase (i.e., C, T or U) typically covalently attaches a 1 position of a pyrimidine to a 1'-position of a 5-carbon sugar.

3. Nucleotides

5

10

15

20

25

30

As used herein, a "nucleotide" refers to a nucleoside further comprising a "backbone moiety". A backbone moiety generally covalently attaches a nucleotide to another molecule comprising a nucleotide, or to another nucleotide to form a nucleic acid. The "backbone moiety" in naturally occurring nucleotides typically comprises a phosphorus moiety, which is covalently attached to a 5-carbon sugar. The attachment of the backbone moiety typically occurs at either the 3'- or 5'-position of the 5-carbon sugar. However, other types of attachments are known in the art, particularly when a nucleotide comprises derivatives or analogs of a naturally occurring 5-carbon sugar or phosphorus moiety.

4. Nucleic Acid Analogs

A nucleic acid may comprise, or be composed entirely of, a derivative or analog of a nucleobase, a nucleobase linker moiety and/or backbone moiety that may be present in a naturally occurring nucleic acid. As used herein a "derivative" refers to a chemically modified or altered form of a naturally occurring molecule, while the terms "mimic" or "analog" refer to a molecule that may or may not structurally resemble a naturally occurring molecule or moiety, but possesses similar functions. As used herein, a "moiety" generally refers to a smaller chemical or molecular component of a larger chemical or molecular structure. Nucleobase, nucleoside and nucleotide analogs or derivatives are well known in the art, and have been described (see for example, Scheit, 1980, incorporated herein by reference).

Additional non-limiting examples of nucleosides, nucleotides or nucleic acids comprising 5-carbon sugar and/or backbone moiety derivatives or analogs, include those in U.S. Patent No. 5,681,947 which describes oligonucleotides comprising purine derivatives that form triple helixes with and/or prevent expression of dsDNA; U.S. Patents 5,652,099 and 5,763,167 which describe nucleic acids incorporating fluorescent analogs of nucleosides found in DNA or RNA, particularly for use as fluorescent nucleic acids probes; U.S. Patent 5,614,617 which describes oligonucleotide analogs with substitutions on pyrimidine rings that possess enhanced nuclease stability, U.S. Patents 5,670,663, 5,872,232 and 5,859,221 which describe oligonucleotide

analogs with modified 5-carbon sugars (i.e., modified 2'-deoxyfuranosyl moieties) used in nucleic acid detection: U.S. Patent 5,446,137 which describes oligonucleotides comprising at least one 5-carbon sugar moiety substituted at the 4' position with a substituent other than hydrogen that can be used in hybridization assays; U.S. Patent 5,886,165 which describes oligonucleotides with both deoxyribonucleotides with 3'-5' internucleotide linkages and ribonucleotides with 2'-5' internucleotide linkages; U.S. Patent 5,714,606 which describes a modified internucleotide linkage wherein a 3'-position oxygen of the internucleotide linkage is replaced by a carbon to enhance the nuclease resistance of nucleic acids; U.S. Patent 5,672,697 which describes oligonucleotides containing one or more 5' methylene phosphonate internucleotide linkages that enhance nuclease resistance; U.S. Patents 5,466,786 and 5,792,847 which describe the linkage of a substituent moiety, which may comprise a drug or label to the 2' carbon of an oligonucleotide to provide enhanced nuclease stability and ability to deliver drugs or detection moieties; U.S. Patent 5,223,618 which describes oligonucleotide analogs with a 2 or 3 carbon backbone linkage attaching the 4' position and 3' position of adjacent 5-carbon sugar mojety to enhanced cellular uptake, resistance to nucleases and hybridization to target RNA; U.S. Patent 5,470,967 which describes oligonucleotides comprising at least one sulfamate or sulfamide internucleotide linkage that are useful as nucleic acid hybridization probe; U.S. Patents 5,378,825, 5,777,092, 5,623,070, 5,610,289 and 5,602,240 which describe oligonucleotides with three or four atom linker moiety replacing phosphodiester backbone mojety used for improved nuclease resistance, cellular uptake and regulating RNA expression; U.S. Patent 5.858.988 which describes hydrophobic carrier agent attached to the 2'-O position of oligonucleotides to enhanced their membrane permeability and stability; U.S. Patent 5,214,136, which describes oligonucleotides conjugated to anthraquinone at the 5' terminus that possess enhanced hybridization to DNA or RNA; enhanced stability to nucleases; U.S. Patent 5,700,922 which describes PNA-DNA-PNA chimeras wherein the DNA comprises 2'-deoxy-erythropentofuranosyl nucleotides for enhanced nuclease resistance, binding affinity, and ability to activate RNase H; and U.S. Patent 5,708,154 which describes RNA linked to a DNA to form a DNA-RNA hybrid. Other analogs that may be used with compositions of the invention include U.S. Patent 5,216,141 (discussing oligonucleotide analogs containing sulfur linkages), U.S. Patent 5.432.272 (concerning oligonucleotides having nucleotides with heterocyclic bases), and U.S. Patents 6,001,983, 6,037,120, 6,140,496 (involving oligonucleotides with non-standard bases), all of which are incorporated by reference.

10

20

25

30

5. Polyether and Peptide Nucleic Acids and Locked Nucleic Acids

In certain embodiments, it is contemplated that a nucleic acid comprising a derivative or analog of a nucleoside or nucleotide may be used in the methods and compositions of the invention. A non-limiting example is a "polyether nucleic acid", described in U.S. Patent Serial No. 5,908,845, incorporated herein by reference. In a polyether nucleic acid, one or more nucleobases are linked to chiral carbon atoms in a polyether backbone.

Another non-limiting example is a "peptide nucleic acid", also known as a "PNA", "peptide-based nucleic acid analog" or "PENAM", described in U.S. Patent Serial Nos. 5,786,461, 5891,625, 5,773,571, 5,766,855, 5,736,336, 5,719,262, 5,714,331, 5,539,082, and WO 92/20702, each of which is incorporated herein by reference. Peptide nucleic acids generally have enhanced sequence specificity, binding properties, and resistance to enzymatic degradation in comparison to molecules such as DNA and RNA (Egholm et al., 1993; PCT/EP/01219). A peptide nucleic acid generally comprises one or more nucleotides or nucleosides that comprise a nucleobase moiety, a nucleobase linker moiety that is not a 5-carbon sugar, and/or a backbone moiety that is not a phosphate backbone moiety. Examples of nucleobase linker moieties described for PNAs include aza nitrogen atoms, amido and/or ureido tethers (see for example, U.S. Patent No. 5,539,082). Examples of backbone moieties described for PNAs include an aminoethylglycine, polyamide, polyethyl, polythioamide, polysulfinamide or polysulfonamide backbone moiety.

10

15

20

25

30

In certain embodiments, a nucleic acid analogue such as a peptide nucleic acid may be used to inhibit nucleic acid amplification, such as in PCR, to reduce false positives and discriminate between single base mutants, as described in U.S. Patent Serial No. 5,891,625. Other modifications and uses of nucleic acid analogs are known in the art, and are encompassed by the bridging and capture nucleic acids of the invention. In a non-limiting example, U.S. Patent 5,786,461 describes PNAs with amino acid side chains attached to the PNA backbone to enhance solubility of the molecule. In another example, the cellular uptake property of PNAs is increased by attachment of a lipophilic group. Several alkylamino moieties used to enhance cellular uptake of a PNA are described in U.S. Patent Nos. 5,766,855, 5,719,262, 5,714,331 and 5,736,336, which describe PNAs comprising naturally and non-naturally occurring nucleobases and alkylamine side chains that provide improvements in sequence specificity, solubility and/or binding affinity relative to a naturally occurring nucleic acid.

5

10

15

20

25

30

Another non-limiting example is a locked nucleic acid or "LNA." An LNA monomer is a bicyclic compound that is structurally similar to RNA nucleosides. LNAs have a furanose conformation that is restricted by a methylene linker that connects the 2'-O position to the 4'-C position, as described in Koshkin et al, 1998a and 1998b and Wahlestedt et al., 2000.

6. Preparation of Nucleic Acids

A nucleic acid may be made by any technique known to one of ordinary skill in the art, such as for example, chemical synthesis, enzymatic production or biological production. Non-limiting examples of a synthetic nucleic acid (e.g., a synthetic oligonucleotide), include a nucleic acid made by in vitro chemical synthesis using phosphotriester, phosphite or phosphoramidite chemistry and solid phase techniques such as described in EP 266,032, incorporated herein by reference, or via deoxynucleoside H-phosphonate intermediates as described by Froehler et al., 1986 and U.S. Patent No. 5,705,629, each incorporated herein by reference. In the methods of the present invention, one or more oligonucleotide may be used. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents. 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244, each of which is incorporated herein by reference.

A non-limiting example of an enzymatically produced nucleic acid include one produced by enzymes in amplification reactions such as PCR™ (see for example, U.S. Patent 4,683,202 and U.S. Patent 4,682,195, each incorporated herein by reference), or the synthesis of an oligonucleotide described in U.S. Patent No. 5,645,897, incorporated herein by reference. A non-limiting example of a biologically produced nucleic acid includes a recombinant nucleic acid produced (i.e., replicated) in a living cell, such as a recombinant DNA vector replicated in bacteria (see for example, Sambrook et al. 1989, incorporated herein by reference).

7. Purification of Nucleic Acids

A nucleic acid may be purified on polyacrylamide gels, cesium chloride centrifugation gradients, or by any other means known to one of ordinary skill in the art (see for example, Sambrook $et\ al.$, 1989, incorporated herein by reference).

In certain aspect, the present invention concerns a nucleic acid that is an isolated nucleic acid. As used herein, the term "isolated nucleic acid" refers to a nucleic acid molecule (e.g., an RNA or DNA molecule) that has been isolated free of, or is otherwise free of, the bulk of the

total genomic and transcribed nucleic acids of one or more cells. In certain embodiments, "isolated nucleic acid" refers to a nucleic acid that has been isolated free of, or is otherwise free of, bulk of cellular components or in vitro reaction components such as for example, macromolecules such as lipids or proteins, small biological molecules, and the like.

8. Nucleic Acid Segments

5

10

20

25

30

In certain embodiments, the nucleic acid comprises a nucleic acid segment. As used herein, the term "nucleic acid segment," are smaller fragments of a nucleic acid, such as for non-limiting example, those that correspond to targeted, targeting, bridging, and capture regions. Thus, a "nucleic acid segment" may comprise any part of a gene sequence, of from about 2 nucleotides to the full length of a targeted nucleic acid, capture nucleic acid, or bridging nucleic acid.

Various nucleic acid segments may be designed based on a particular nucleic acid sequence, and may be of any length. By assigning numeric values to a sequence, for example, the first residue is 1, the second residue is 2, etc., an algorithm defining all nucleic acid segments can be created:

15 n to n + v

where n is an integer from 1 to the last number of the sequence and y is the length of the nucleic acid segment minus one, where n + y does not exceed the last number of the sequence. Thus, for a 10-mer, the nucleic acid segments correspond to bases 1 to 10, 2 to 11, 3 to 12 ... and so on. For a 15-mer, the nucleic acid segments correspond to bases 1 to 15, 2 to 16, 3 to 17 ... and so on. For a 20-mer, the nucleic segments correspond to bases 1 to 20, 2 to 21, 3 to 22 ... and so on. In certain embodiments, the nucleic acid segment may be a probe or primer. As used herein, a "probe" generally refers to a nucleic acid used in a detection method or composition. As used herein, a "primer" generally refers to a nucleic acid used in an extension or amplification method or composition.

9. Nucleic Acid Complements

The present invention also encompasses a nucleic acid that is complementary to a other nucleic acids of the invention and targeted nucleic acids. More specifically, a targeting region in a bridging nucleic acid is complementary to the targeted region of the targeted nucleic acid and a bridging region of the bridging nucleic acid is complementary to a capture region of a capture nucleic acid. In particular embodiments the invention encompasses a nucleic acid or a nucleic

10

15

20

25

30

acid segment identical or complementary to all or part of the sequences set forth in SEQ ID NOS:1-92. A nucleic acid is "complement(s)" or is "complementary" to another nucleic acid when it is capable of base-pairing with another nucleic acid according to the standard Watson-Crick, Hoogsteen or reverse Hoogsteen binding complementarity rules. Unless otherwise specified, a nucleic acid region is "complementary" to another nucleic acid region if there is at least 70, 80%, 90% or 100% Watson-Crick base-pairing (A:T or A:U, C:G) between or between at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500 or more contiguous nucleic acid bases of the regions. As used herein "another nucleic acid" may refer to a separate molecule or a spatial separated sequence of the same molecule.

As used herein, the term "complementary" or "complement(s)" also refers to a nucleic acid comprising a sequence of consecutive nucleobases or semiconsecutive nucleobases (e.g., one or more nucleobase moieties are not present in the molecule) capable of hybridizing to another nucleic acid strand or duplex even if less than all the nucleobases do not base pair with a counterpart nucleobase. In certain embodiments, a "complementary" nucleic acid comprises a sequence in which at least 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 89%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%, and any range derivable therein, of the nucleobase sequence is capable of base-pairing with a single or double stranded nucleic acid molecule during hybridization, as described in the Examples. In certain embodiments, the term "complementary" refers to a nucleic acid that may hybridize to another nucleic acid strand or duplex under conditions described in the Examples, as would be understood by one of ordinary skill in the art.

In certain embodiments, a "partly complementary" nucleic acid comprises a sequence that may hybridize in low stringency conditions to a single or double stranded nucleic acid, or contains a sequence in which less than about 70% of the nucleobase sequence is capable of base-pairing with a single or double stranded nucleic acid molecule during hybridization.

10. Hybridization

As used herein, "hybridization", "hybridizes" or "capable of hybridizing" is understood to mean the forming of a double or triple stranded molecule or a molecule with partial double or triple stranded nature. The term "anneal" as used herein is synonymous with "hybridize." The term "hybridization", "hybridize(s)" or "capable of hybridizing" encompasses the terms "stringent condition(s)" or "high stringency" and the terms "low stringency" or "low stringency condition(s)."

5

15

20

25

30

As used herein "stringent condition(s)" or "high stringency" are those conditions that allow hybridization between or within one or more nucleic acid strand(s) containing complementary sequence(s), but precludes hybridization of random sequences. Stringent conditions tolerate little, if any, mismatch between a nucleic acid and a target strand. Such conditions are well known to those of ordinary skill in the art, and are preferred for applications requiring high selectivity. Non-limiting applications include isolating a nucleic acid, such as a gene or a nucleic acid segment thereof, or detecting at least one specific mRNA transcript or a nucleic acid segment thereof, and the like.

Stringent conditions may comprise low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.15 M NaCl at temperatures of about 50°C to about 70°C. Alternatively, stringent conditions may be determined largely by temperature in the presence of a TMAC solution with a defined molarity such as 3M TMAC. For example, in 3 M TMAC, stringent conditions include the following: for complementary nucleic acids with a length of 15 bp, a temperature of 45°C to 55°C; for complementary nucleotides with a length of 27 bases, a temperature of 65°C to 75°C; and, for complementary nucleotides with a length of >200 nucleotides, a temperature of 90°C to 95°C. The publication of Wood et al., 1985, which is specifically incorporated by reference, provides examples of these parameters. It is understood that the temperature and ionic strength of a desired stringency are determined in part by the length of the particular nucleic acid(s), the length and nucleobase content of the target sequence(s), the charge composition of the nucleic acid(s), and to the presence or concentration of formamide, tetramethylammonium chloride or other solvent(s) in a hybridization mixture.

It is also understood that these ranges, compositions and conditions for hybridization are mentioned by way of non-limiting examples only, and that the desired stringency for a particular hybridization reaction is often determined empirically by comparison to one or more positive or negative controls. Depending on the application envisioned it is preferred to employ varying conditions of hybridization to achieve varying degrees of selectivity of a nucleic acid towards a target sequence. In a non-limiting example, identification or isolation of a related target nucleic

acid that does not hybridize to a nucleic acid under stringent conditions may be achieved by hybridization at low temperature and/or high ionic strength. Such conditions are termed "low stringency" or "low stringency conditions", and non-limiting examples of low stringency include hybridization performed at about 0.15 M to about 0.9 M NaCl at a temperature range of about 50°C. Of course, it is within the skill of one in the art to further modify the low or high stringency conditions to suite a particular application.

11. Oligonucleotide Synthesis

15

20

25

Oligonucleotide synthesis is performed according to standard methods. See, for example, Itakura and Riggs (1980). Additionally, U.S. Patent 4,704,362; U.S. Patent 5,221,619, U.S. Patent 5,583,013 each describe various methods of preparing synthetic structural genes.

Oligonucleotide synthesis is well known to those of skill in the art. Various different mechanisms of oligonucleotide synthesis have been disclosed in for example, U.S. Patents. 4,659,774, 4,816,571, 5,141,813, 5,264,566, 4,959,463, 5,428,148, 5,554,744, 5,574,146, 5,602,244, each of which is incorporated herein by reference.

Basically, chemical synthesis can be achieved by the diester method, the triester method polynucleotides phosphorylase method and by solid-phase chemistry. These methods are discussed in further detail below.

Diester method. The diester method was the first to be developed to a usable state, primarily by Khorana and co-workers. (Khorana, 1979). The basic step is the joining of two suitably protected deoxynucleotides to form a dideoxynucleotide containing a phosphodiester bond. The diester method is well established and has been used to synthesize DNA molecules (Khorana, 1979).

Triester method. The main difference between the diester and triester methods is the presence in the latter of an extra protecting group on the phosphate atoms of the reactants and products (Itakura et al., 1975). The phosphate protecting group is usually a chlorophenyl group, which renders the nucleotides and polynucleotide intermediates soluble in organic solvents. Therefore purification's are done in chloroform solutions. Other improvements in the method include (i) the block coupling of trimers and larger oligomers, (ii) the extensive use of high-

performance liquid chromatography for the purification of both intermediate and final products, and (iii) solid-phase synthesis.

Polynucleotide phosphorylase method. This is an enzymatic method of DNA synthesis that can be used to synthesize many useful oligodeoxynucleotides (Gillam et al., 1978; Gillam et al., 1979). Under controlled conditions, polynucleotide phosphorylase adds predominantly a single nucleotide to a short oligodeoxynucleotide. Chromatographic purification allows the desired single adduct to be obtained. At least a trimer is required to start the procedure, and this primer must be obtained by some other method. The polynucleotide phosphorylase method works and has the advantage that the procedures involved are familiar to most biochemists.

Solid-phase methods. Drawing on the technology developed for the solid-phase synthesis of polypeptides, it has been possible to attach the initial nucleotide to solid support material and proceed with the stepwise addition of nucleotides. All mixing and washing steps are simplified, and the procedure becomes amenable to automation. These syntheses are now routinely carried out using automatic DNA synthesizers.

Phosphoramidite chemistry (Beaucage, and Lyer, 1992) has become by far the most widely used coupling chemistry for the synthesis of oligonucleotides. As is well known to those skilled in the art, phosphoramidite synthesis of oligonucleotides involves activation of nucleoside phosphoramidite monomer precursors by reaction with an activating agent to form activated intermediates, followed by sequential addition of the activated intermediates to the growing oligonucleotide chain (generally anchored at one end to a suitable solid support) to form the oligonucleotide product.

12. Expression Vectors

10

15

20

25

Other ways of creating nucleic acids of the invention include the use of a recombinant vector created through the application of recombinant nucleic acid technology known to those of skill in the art or as described herein. A recombinant vector may comprise a bridging or capture nucleic acid, particularly one that is a polynucleotide, as opposed to an oligonucleotide. An expression vector can be used create nucleic acids that are lengthy, for example, containing multiple targeting regions or relatively lengthy targeting regions, such as those greater than 100 residues in length.

The term "vector" is used to refer to a carrier nucleic acid molecule into which a nucleic acid sequence can be inserted for introduction into a cell where it can be replicated. A nucleic acid sequence can be "exogenous," which means that it is foreign to the cell into which the vector is being introduced or that the sequence is homologous to a sequence in the cell but in a position within the host cell nucleic acid in which the sequence is ordinarily not found. Vectors include plasmids, cosmids, viruses (bacteriophage, animal viruses, and plant viruses), and artificial chromosomes (e.g., YACs). One of skill in the art would be well equipped to construct a vector through standard recombinant techniques (see, for example, Sambrook et al., 2001 and Ausubel et al., 1994, both incorporated herein by reference).

The term "expression vector" refers to any type of genetic construct comprising a nucleic acid coding for a RNA capable of being transcribed. Expression vectors can contain a variety of "control sequences," which refer to nucleic acid sequences necessary for the transcription and possibly translation of an operable linked coding sequence in a particular host cell. In addition to control sequences that govern transcription (promoters and enhancers) and translation, vectors and expression vectors may contain nucleic acid sequences that serve other functions as well that are well known to those of skill in the art, such as screenable and selectable markers, ribosome binding site, multiple cloning sites, splicing sites, poly A sequences, origins of replication, and other sequences that allow expression in different hosts.

10

15

20

25

Numerous expression systems exist that comprise at least a part or all of the compositions discussed above. Prokaryote- and/or eukaryote-based systems can be employed for use with the present invention to produce nucleic acid sequences, or their cognate polypeptides, proteins and pentides. Many such systems are commercially and widely available.

The nucleotide and protein, polypeptide and peptide sequences for various genes have been previously disclosed, and may be found at computerized databases known to those of ordinary skill in the art. For example, the nucleotide sequences of rRNAs of various organisms are readily available. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (http://www.ncbi.nlm.nih.gov/). The coding regions for all or part of these known genes may be amplified and/or expressed using the techniques disclosed herein or by any technique that would be know to those of ordinary skill in the art.

13. Nucleic Acid Arrays

10

15

20

25

30

Because the present invention provides efficient methods of enriching in mRNA, which can be used to make cDNA, the present invention extends to the use of cDNAs with arrays. The term "array" as used herein refers to a systematic arrangement of nucleic acid. For example, a cDNA population that is representative of a desired source (e.g., human adult brain) is divided up into the minimum number of pools in which a desired screening procedure can be utilized to detect a cDNA and which can be distributed into a single multi-well plate. Arrays may be of an aqueous suspension of a cDNA population obtainable from a desired mRNA source, comprising: a multi-well plate containing a plurality of individual wells, each individual well containing an aqueous suspension of a different content of a cDNA population. The cDNA population may include cDNA of a predetermined size. Furthermore, the cDNA population in all the wells of the plate may be representative of substantially all mRNAs of a predetermined size from a source. Examples of arrays, their uses, and implementation of them can be found in U.S. Patent Nos. 6,329,209, 6,329,140, 6,324,479, 6,322,971, 6,316,193, 6,309,823, 5,412,087, 5,445,934, and 5,744,305, which are herein incorporated by reference.

The number of cDNA clones array on a plate may vary. For example, a population of cDNA from a desired source can have about 200,000-6,000,000 cDNAs, about 200,000-2,000,000, 300,000-700,000, about 400,000-600,000, or about 500,000 cDNAs, and combinations thereof. Such a population can be distributed into a small set of multi-well plates, such as a single 96-well plate or a single 384-well plate. For instance, when about 1000-10,000 cDNAs, preferably about 3,500-7,000, more preferably about 5,000, from a population are present in a single well of a 96-well or 384-well plate, PCR can be utilized to clone a single, larget zene using a set of primers.

The term a "nucleic acid array" refers to a plurality of target elements, each target element comprising one or more nucleic acid molecules immobilized on one or more solid surfaces to which sample nucleic acids can be hybridized. The nucleic acids of a target element can contain sequence(s) from specific genes or clones, e.g. from the regions identified here. Other target elements will contain, for instance, reference sequences. Target elements of various dimensions can be used in the arrays of the invention. Generally, smaller, target elements are preferred. Typically, a target element will be less than about 1 cm in diameter. Generally element sizes are from 1 μ m to about 3 mm, between about 5 μ m and about 1 mm. The target elements

of the arrays may be arranged on the solid surface at different densities. The target element densities will depend upon a number of factors, such as the nature of the label, the solid support, and the like. One of skill will recognize that each target element may comprise a mixture of nucleic acids of different lengths and sequences. Thus, for example, a target element may contain more than one copy of a cloned piece of DNA, and each copy may be broken into fragments of different lengths. The length and complexity of the nucleic acid fixed onto the target element is not critical to the invention. One of skill can adjust these factors to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among different genes or genomic locations. In various embodiments, target element sequences will have a complexity between about 1 kb and about 1 Mb, between about 10 kb to about 500 kb, between about 200 to about 500 kb, and from about 50 kb to about 150 kb.

10

15

20

25

30

Microarrays are known in the art and consist of a surface to which probes that correspond in sequence to gene products (e.g., cDNAs, mRNAs, cRNAs, polypeptides, and fragments thereof), can be specifically hybridized or bound at a known position. In one embodiment, the microarray is an array (i.e., a matrix) in which each position represents a discrete binding site for a product encoded by a gene (e.g., a protein or RNA), and in which binding sites are present for products of most or almost all of the genes in the organism's genome. In a preferred embodiment, the "binding site" (hereinafter, "site") is a nucleic acid or nucleic acid analogue to which a particular cognate cDNA can specifically hybridize. The nucleic acid or analogue of the binding site can be, e.g., a synthetic oligomer, a full-length cDNA, a less-than full length cDNA, or a gene fragment.

A microarray may contains binding sites for products of all or almost all genes in the target organism's genome, but such comprehensiveness is not necessarily required. Usually the microarray will have binding sites corresponding to at least about 50% of the genes in the genome, often at least about 75%, more often at least about 85%, even more often more than about 90%, and most often at least about 99%. Preferably, the microarray has binding sites for genes relevant to the action of a drug of interest or in a biological pathway of interest. A "gene" is identified as an open reading frame (ORF) of preferably at least 50, 75, or 99 amino acids from which a messenger RNA is transcribed in the organism (e.g., if a single cell) or in some cell in a multicellular organism. The number of genes in a genome can be estimated from the number

of mRNAs expressed by the organism, or by extrapolation from a well-characterized portion of the genome. When the genome of the organism of interest has been sequenced, the number of ORFs can be determined and mRNA coding regions identified by analysis of the DNA sequence.

The nucleic acid or analogue are attached to a solid support, which may be made from glass, plastic (e.g., polypropylene, nylon), polyacrylamide, nitrocellulose, or other materials. A preferred method for attaching the nucleic acids to a surface is by printing on glass plates, as is described generally by Schena et al., 1995a. See also DeRisi et al., 1996; Shalon et al., 1996; Schena et al., 1995b. Each of these articles is incorporated by reference in its entirety.

Other methods for making microarrays, e.g., by masking (Maskos et al., 1992), may also be used. In principal, any type of array, for example, dot blots on a nylon hybridization membrane (see Sambrook et al., 1989, which is incorporated in its entirety for all purposes), could be used, although, as will be recognized by those of skill in the art, very small arrays will be preferred because hybridization volumes will be smaller.

10

15

20

25

30

Labeled cDNA is prepared from mRNA by oligo dT-primed or random-primed reverse transcription, both of which are well known in the art (see e.g., Klug et al., 1987). Reverse transcription may be carried out in the presence of a dNTP conjugated to a detectable label, most preferably a fluorescently labeled dNTP. Alternatively, isolated mRNA can be converted to labeled antisense RNA synthesized by in vitro transcription of double-stranded cDNA in the presence of labeled dNTPs (Lockhart et al., 1996, which is incorporated by reference in its entirety for all purposes). In alternative embodiments, the cDNA or RNA probe can be synthesized in the absence of detectable label and may be labeled subsequently, e.g., by incorporating biotinylated dNTPs or rNTP, or some similar means (e.g., photo-cross-linking a psoralen derivative of biotin to RNAs), followed by addition of labeled streptavidin (e.g., phycocrythrin-conjugated streptavidin) or the equivalent.

Fluorescently-labeled probes can be used, including suitable fluorophores such as fluorescein, lissamine, phycoerythrin, rhodamine (Perkin Elmer Cetus), Cy2, Cy3, Cy3.5, Cy5, Cy5.5, Cy7, FluorX (Amersham) and others (see, e.g., Kricka, 1992). It will be appreciated that pairs of fluorophores are chosen that have distinct emission spectra so that they can be easily distinguished. In another embodiment, a label other than a fluorescent label is used. For example, a radioactive label, or a pair of radioactive labels with distinct emission spectra, can be used (see

51

Zhao et al., 1995; Pietu et al., 1996). However, because of scattering of radioactive particles, and the consequent requirement for widely spaced binding sites, use of radioisotopes is a lesspreferred embodiment.

In one embodiment, labeled cDNA is synthesized by incubating a mixture containing 0.5 mM dGTP, dATP and dCTP plus 0.1 mM dTTP plus fluorescent deoxyribonucleotides (e.g., 0.1 mM Rhodamine 110 UTP (Perken Elmer Cetus) or 0.1 mM Cy3 dUTP (Amersham)) with reverse transcriptase (e.g., SuperScriptTM, Invitrogen Inc.) at 42°C for 60 min.

III. Methods for Isolating and Depleting Targeted Nucleic Acids

10

15

20

25

30

Methods of the invention involve preparing a sample comprising a targeted nucleic acid, preparing a bridging nucleic acid, preparing a capture nucleic acid, incubating the sample with the bridging nucleic acid, incubating the sample with a capture nucleic acid, incubating the bridging nucleic acid with the capture nucleic acid, incubating compounds under conditions allowing for hybridization among complementary regions, washing the sample and/or the capture and/or bridging nucleic acids, and isolating the capture nucleic acids and any accompanying compounds (compounds that bind or hybridize directly or indirectly to the capture nucleic acids). Steps of the invention are not required to be in a particular order and thus, the invention covers methods in which the order of the steps varies.

Hybridization conditions are discussed earlier. Wash conditions may involve temperatures between 20°C and 75°C, between 25°C and 70°C, between 30°C and 60°C, between 40°C and 55°C, between 45°C and 50°C, or at temperatures within the ranges specified.

Buffer conditions for hybridization of nucleic acid compositions are well known to those of skill in the art. It is specifically contemplated that isostabilizing agents may be employed in hybridization and wash buffers in methods of the invention. U.S. Ser. No. 09/854,412 describes the use of tetramethylammonium chloride (TMAC) and tetraethylammonium chloride (TEAC) in such buffers; this application is specifically incorporated by reference herein. The concentration of an isostabilizing agent in a hybridization (binding) buffer may be between about 1.0 M and about 5.0 M, is about 4.0 M, or is about 2.0 M. Also specifically contemplated is a wash solution with an isostabilizing agent concentration of between about 0.1 M and 3.0 M, including 0.1 M increments within the range. Wash buffers may or may not contain Tris. However, in

some embodiments of the invention, the wash solution consists of water and no other salts or buffers. In some embodiments of the invention, the hybridizing or wash buffer may include guanidinium isothicoyanate, though in some embodiments this chemical is specifically contemplated to be absent. The concentration of guanidinium may be between about 0.4 M and about 3.0 M

A solution or buffer to elute targeted nucleic acids from the hybridizing nucleic acids (indirect or direct) may be implemented in some kits and methods of the invention. The elution buffer or solution can be an aqueous solution lacking salt, such as TE or water. Elution may occur at room temperature or it may occur at temperatures between 15°C and 100°C, between 20°C and 95°C, between 25°C and 90°C, between 30°C and 85°C, between 35°C and 80°C, between 40°C and 75°C, between 45°C and 70°C, between 50°C and 65°C, between 55°C and 60°C, or at temperatures within the ranges specified.

A. Quantitation of RNA

15

20

25

1. Assessing RNA yield by UV absorbance

The concentration and purity of RNA can be determined by diluting an aliquot of the preparation (usually a 1:50 to 1:100 dilution) in TE (10 mM Tris-HCl pH 8, 1 mM EDTA) or water, and reading the absorbance in a spectrophotometer at 260 nm and 280 nm.

An A_{260} of 1 is equivalent to 40 μ g RNA/ml. The concentration (μ g/ml) of RNA is therefore calculated by multiplying the A_{260} X dilution factor X 40 μ g/ml. The following is a typical example:

The typical yield from 10 μ g total RNA is 3 - 5 μ g. If the sample is re-suspended in 25 μ l, this means that the concentration will vary between 120 ng/ μ l and 200 ng/ μ l. One μ l of the prep is diluted 1:50 into 49 μ l of TE. The A₂₆₀ = 0.1. RNA concentration = 0.1 X 50 X 40 μ g/ml = 200 μ g/ml or 0.2 μ g/ μ l. Since there are 24 μ l of the prep remaining after using 1 μ l to measure the concentration, the total amount of remaining RNA is 24 μ l X 0.2 μ g/ μ l = 4.8 μ g.

2. Assessing RNA yield with RiboGreen®

Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation can be employed to measure RNA concentration.

B. Denaturing Agarose Gel Electrophoresis

Many mRNAs form extensive secondary structure. Ribosomal RNA depletion may be evaluated by agarose gel electrophoresis. Because of this, it is best to use a denaturing gel system to analyze RNA samples. A positive control should be included on the gel so that any unusual results can be attributed to a problem with the gel or a problem with the RNA under analysis. RNA molecular weight markers, an RNA sample known to be intact, or both, can be used for this purpose. It is also a good idea to include a sample of the starting RNA that was used in the enrichment procedure.

Ambion's NorthernMax™ reagents for Northern Blotting include everything needed for denaturing agarose gel electrophoresis. These products are optimized for ease of use, safety, and low background, and they include detailed instructions for use. An alternative to using the NorthernMax reagents is to use a procedure described in "Current Protocols in Molecular Biology", Section 4.9 (Ausubel et al., eds.), hereby incorporated by reference. It is more difficult and time-consuming than the Northern-Max method, but it gives similar results.

C. Agilent 2100 Bioanalyzer

15

20

25

Evaluating rRNA Removal with the RNA 6000 LabChip

An effective method for evaluating rRNA removal utilizes RNA analysis with the Caliper RNA 6000 LabChip Kit and the Agilent 2100 Bioanalayzer. Follow the instructions provided with the RNA 6000 LabChip Kit for RNA analysis. This system performs best with RNA solutions at concentrations between 50 and 250 ng/µl. Loading 1 µl of a typical enriched RNA sample is usually adequate for good performance.

2. Expected Results

In enriched mRNA samples from prokaryotes, the 16S and 23S rRNA peaks will be absent or present in only very small amounts. The peak calling feature of the software may fail to identify the peaks containing small quantities of leftover 16S and 23S rRNAs. A peak corresponding to 5S and tRNAs may be present depending on how the total RNA was initially purified. If RNA was purified by a glass fiber filter method prior to enrichment, this peak will be smaller. The size and shape of the 5S rRNA-tRNA peak is unchanged by some embodiments.

IV. KITS

10

15

20

25

Any of the compositions described herein may be comprised in a kit. In a non-limiting example, a bridging nucleic acid and a capture nucleic acid may be comprised in a kit. The kits will thus comprise, in suitable container means, a bridging nucleic acid and a capture nucleic of the present invention. It may also include one or more buffers, such as hybridization buffer or a wash buffer, compounds for preparing the sample, and components for isolating the capture nucleic acid via the nonreacting structure. Other kits of the invention may include components for making a nucleic acid array, and thus, may include, for example, a solid support.

The kits may comprise suitably aliquoted nucleic acid compositions of the present invention, whether labeled or unlabeled, as may be used to isolate, deplete, or separate a targeted nucleic acid. The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit (bridging and capture nucleic acids may be packaged together), the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred.

However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means.

The container means will generally include at least one vial, test tube, flask, bottle, syringe and/or other container means, into which the nucleic acid formulations are placed,

preferably, suitably allocated. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent.

The kits of the present invention will also typically include a means for containing the vials in close confinement for commercial sale, such as, e.g., injection and/or blow-molded plastic containers into which the desired vials are retained.

Such kits may also include components that facilitate isolation of the targeting molecule, such as filters, beads, or a magnetic stand. Such kits generally will comprise, in suitable means, distinct containers for each individual reagent or solution as well as for the targeting agent.

A kit will also include instructions for employing the kit components as well the use of
any other reagent not included in the kit. Instructions may include variations that can be
implemented.

Kits of the invention may also include one or more of the following, in addition to a capture nucleic acid and a bridging nucleic acid:

- Control RNA (E. coli or other appropriate RNA);
- 15 2) Nuclease-free water;
 - 3) RNase-free containers, such as 1.5 ml tubes;
 - RNase-free elution tubes;
 - glycogen;
 - 6) ethanol;
- 20 7) sodium acetate;
 - ammonium acetate;
 - 9) magnetic stand or other magnetic field;
 - agarose;
 - 11) nucleic acid size marker;
- 25 12) RNase-free tube tips;
 - 13) and RNase or DNase inhibitors.

IV. Examples

10

15

25

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Furthermore, these examples are provided as one of many ways of implementing the claimed method and using the compositions of the invention. It is contemplated that the invention is not limited to the specific conditions set forth below, but that the conditions below provide examples of how to implement the invention.

EXAMPLE 1:

Materials

The following materials were used in the methods described herein for the selective removal of 16S and 23S rRNA and/or 18S and 28S rRNA, and hence mRNA enrichment, from total RNA. All steps are performed at room temperature unless otherwise indicated.

1. Bridging Nucleic Acids

In the following examples, the bridging regions are the poly-A stretches in the respective oligonucleotides.

Targeting regions for prokaryotic 16S and 23S rRNAs were designed based on a sequence comparison of different rRNAs from different bacteria to E. coli rRNA with MegAlign sequence analysis software version 4.05 from DNA Star, Incorporated (FIG. 2). The targeting regions are shown, in the examples below, 3' of the bridging regions. Thus, the targeting region encompasses the remaining, non-bridging region of each molecule described below. SEQ ID NOs are provided for the targeting regions of the bridging nucleic acids provided below (i.e., sequence of bridging regions not included in SEQ ID NO.).

16S prokaryotic rRNA bridging oligonucleotides

d16S-358 (SEQ ID NO:1)

5'-AAAAAAAAAAAAAAAAAAAAACTGCTGCCTCCCGTAGGAGTCT-3'
d168-537 (SEQ ID NO:2) 5'-AAAAAAAAAAAAAAAAAAACGTATTACCGCGGCTGCTGGCAC-3'
d16S-548 (SEQ ID NO:3) 5'-AAAAAAAAAAAAAAAAAAAAACGCCCAGTAATTCCGATTAACGC-3'
d16S-807 (SEQ ID NO:4) 5'-AAAAAAAAAAAAAAAAAAAATGGACTACCAGGGTATCTAATCC-3'
d16S-1092 (SEQ ID NO:5) 5'-AAAAAAAAAAAAAAAAAAGGGTTGCGCTCGTTGCGGGACTT-3'
d16S-3' (SEQ ID NO:6) 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
23S prokaryotic rRNA bridging oligonucleotides
d23S-488 (SEQ ID NO:7) 5'-AAAAAAAAAAAAAAAAAAAAGGTTCTTTTTCACTCCCCTCGCC-3'
d23S-581 (SEQ ID NO:8) 5'-AAAAAAAAAAAAAAAAAAAAAAAAAGACCCATTATACAAAAGGTACGC-3'
d23S-1118 (SEQ ID NO:9) 5'-AAAAAAAAAAAAAAAAAAAGCCCCGTTACATCTTCCGCGCAG-3'
d23S-1926 (SEQ ID NO:10) 5'-AAAAAAAAAAAAAAAAAAAACGACAAGGAATTTCGCTACCTTA-3'
d23S-1954 (SEQ ID NO:11) 5'-AAAAAAAAAAAAAAAAAAAAAACTTACCCGACAAGGAATTTCGC-3'
d23S-2511 (SEQ ID NO:12) 5'-AAAAAAAAAAAAAAAAAAAAAAAGAGCCGACATCGAGGTGCCAAAC-3'
d23S-3' (SEQ ID NO:13) 5'-AAAAAAAAAAAAAAAAAAAAAAGGTTAAGCCTCACGGTTCATT-3'
d23S-1704 (SEQ ID NO:15) 5'-AAAAAAAAAAAAAAAAAAAACCCCTTCTCCCGAAGTTACGGGG-3'
<u>d23S-1105</u> (SEQ ID NO:16) 5'-AAAAAAAAAAAAAAAAAAAAAAAGTGAGCTATTACGCTTTCTTT

15

RNA oligo bridging oligonucleotide

r23S-3' (SEQ ID NO:14)
5'-AAAAAAAAAAAAAAAAAAGGUUAAGCGUCACGGUUCAUU-(inverted (dT))-3' (inverted refers to bases attached 3' to 3')

<u>Eukaryotic 18S rRNA bridging oligonucleotides</u>
d18S-3711 (SEQ ID NO:17)
AAA AAA AAA AAA AAA AAA AAA AAA CGG CCG TGC GTA CTT AGA CA

10 d18S-4238 (SEQ ID NO.18)
AAA AAA AAA AAA AAA AAA TGC CCT CCA ATG GAT CCT CGT TA
d18S-5482 (SEQ ID NO.19)
AAA AAA AAA AAA AAA AAA CTA CGG AAA CCT TGT TAC GAC TT

Eukaryotic 28S rRNA bridging oligonucleotides

d288-11599 (SEQ ID NO:20) AAA AAA AAA AAA AAA AAA GAG CAC TGG GCA GAA ATC ACA TC 20

d28S-7979 (SEQ ID NO:21)
AAA AAA AAA AAA AAA AAA GTT TCT TTT CCT CCG CTG ACT AA

d28S-12533 (SEQ ID NO:22)

AAA AAA AAA AAA AAA AAA TCC TCA GCC AAG CAC ATA CAC CA

- Binding Buffer (also referred to as hybridization buffer)
 M TMAC, 10 mM Tris, (pH 7.0)
- Bridging Nucleic Acid Mixture
 Mixtures of 16S, 23S, 18S, and/or 28S bridging oligonucleotides were used. All oligonucleotides were purchased from IDT and purified from polyacrylamide gels.
 - 4. Capture Nucleic Acid (Oligo(dT) MagBeads) Seradyn MGOL #2815-2103.
- 35 5. Wash Solution2 M TMAC, 6.67 mM Tris (pH 7.0) (this is a dilution of binding buffer).

EXAMPLE 2: Methods for rRNA Depletion from Prokaryotic Total RNA

The following methods are provided by way of example for practicing methods of the invention. They have been performed and shown to effect methods of the invention. The invention is not intended to be limited to these protocols, and it is specifically contemplated that variations of the methods below may be employed that fall within the scope of the invention if they effect depletion, isolation, or separation of a targeted nucleic acid, particularly rRNA.

This example demonstrates the depletion of 16S and 23S rRNA from E. coli total RNA.

10 RNA/Bridging Nucleic Acid Mixture Annealing

RNA (10 $\mu g/15$ μl) was added to 200 μl of binding buffer. The bridging nucleic acid mixture consisted of d16S-807 (5 μM), d16S-1092 (5 μM), d23S-1954 (5 μM), d23S-2511 (5 μM). The bridging nucleic acid mixture (4 μl) was added to the RNA and the mixture was incubated at 70°C for 10 minutes and then shifted to 37°C for 30 minutes.

Thirty minutes was found to be an adequate time for the annealing step. Longer time periods can be used with no adverse effects. Between fifteen and 120 minutes have been used successfully in the methods of the invention.

Preparation of Capture Nucleic Acid

Capture nucleic acid (Oligo (dT) MagBeads, Seradyn) in storage buffer was mixed and 50 µl was removed to a separate tube. A magnetic stand was applied to the side of the tube to capture the magnetic beads and the supernatant was removed. The capture nucleic acid was equilibrated one time with distilled, deionized water (50 µl) and once with binding buffer (50 µl). The captured nucleic acid was captured again with a magnetic stand, and the binding buffer wash was removed. The magnetic beads were resuspended in 50 µl of binding buffer.

25 rRNA Capture

15

20

30

Following the 30 minute annealing of RNA with the bridging nucleic acid mixture, the capture nucleic acid was added and the mixture was incubated at room temperature for 15 minutes. A magnetic stand was then applied to the tube to capture the magnetic beads. The supernatant containing mRNA, 5S rRNA, and tRNAs was removed to another tube and saved. An optional washing step was performed next. The magnetic beads were washed with Wash

Solution (100 µl) and captured again. The wash supernatant was removed and added to the original supernatant.

Fifteen minutes was found to be an adequate time for rRNA capture. Longer time periods can be used with no adverse effects. rRNA capture likely occurs rapidly, and capture times of 5 minutes – 60 minutes have been used successfully in the methods of the invention.

Precipitating mRNA

10

15

20

25

mRNA, 5S rRNA, and tRNAs were precipitated by adding 1/10 volume of 3M NaOAc (pH 5.5) and 3 volumes of 100% EtOH and incubating at -20° C for 60 minutes. The precipitated RNA was pelleted in a microfuge, washed with 70% EtOH, and resuspended in TE (oH 8.0).

Analysis of Purified mRNA

Purified mRNA was analyzed with the Caliper RNA 6000 LabChip kit on an Agilent Bioanalyzer. Purified RNA was compared with a control E. coli total RNA sample that was carried through the reaction as described above, except that the Bridging Nucleic Acid Mixture was left out. This assay system uses electrophoretic and electrokinetic separation in a capillary electrophoresis type system. The rRNAs appear as peaks on an electropherogram (FIG. 3). The percentage of a rRNA present in the sample is calculated from the area under the peak.

Under the protocol conditions described above, the 5S + tRNA peak area is essentially the same in the control and in experimental samples. The % of 16S or 23S rRNA removed was calculated using the ratios of 16S_{peak area}/SS_{peak area} and 23S_{peak area}/5S_{peak area}. Enriched and control RNAs with similar 5S + tRNA peak areas were compared.

% 16S rRNA removed =

(16Speak area/5Speak area)no oligos control — (16Speak area/5Speak area)experimental, X 100 (16Speak area/5Speak area)experimental, X 100

A corresponding formula was used to calculate % 23S rRNA removed.

Electropherograms of RNA from a control reaction and from an experimental reaction after ribosomal RNA depletion are shown in FIG. 3 and FIG. 4.

EXAMPLE 3:

Evaluations of Efficacy with Prokaryotic Targets

The materials and methods of Examples 1 and 2 were employed to determine the efficiency of removal of 16S rRNA or 23S rRNA or both from *E. coli* total RNA. Changes in 5 the parameters of the experiments are noted when appropriate. These experiments were performed to evaluate the efficacy of various bridging nucleic acids and reaction conditions.

The following results are from reactions that employed 10 µg of *E. coli* total RNA, 40 pmol of total 16S rRNA bridging nucleic acid, 40 pmol of total 23S rRNA bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1.

Bridging Nucleic	% 16S Removed	% 23S Removed
Acid	average of 2	average of 2
16S/23S	reactions	reactions
d16S-358/d23S-2511	96.48285	89.86496
d16S-537/d23S-1954	97.47974	91.32074
d16S-537/d23S-2511	97.48704	91.216
d16S-807/d23S-1954	95.79126	89.85388
d16S-807/d23S-2511	95.25362	91.06399
d16S-1092/d23S-1118	97.91265	96.50658
d16S-1092/d23S-1954	96.7473	89.40605
d16S-1092/d23S-2511	97.61689	91.5964
d16S-358/d23S-1954	96.74434	88.07242
d16S1092/d23S-1954	97.19134	98.44728
(20 pmol)		
d23S-2511 (20 pmol)		

10

The following results are from reactions that employed 10 µg of E. coli total RNA, 26 pmol of 16S rRNA bridging nucleic acid, 26 pmol of 23S rRNA bridging nucleic acid, and 35 µl of canture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-1092/d23S-1118	97.38534	95.02083
d16S-1092/d23S-1957	97.8291	90.798

5

The following results are from reactions that employed 10 μ g of E.~coli total RNA, 75 pmol of 16S rRNA bridging nucleic acid, 75 pmol of 23S rRNA bridging nucleic acid, and 100 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-1092d23S-1118	99.14812	99.11895
d16S-1092d23S-1954	98.79938	98.45245
d16S-1092d23S-2511	99.00567	98.84033

The following results are from reactions that employed 10 μ g of *E. coli* total RNA, 37.5 pmol of 16S rRNA bridging nucleic acid, 37.5 pmol of 23S rRNA bridging nucleic acid, and 50 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-1092/d23S-1118	98.95563	98.28748
d16S-1092/d23S-1954	97.83593	94.84438

The following results are from reactions that employed 10 μ g of *E. coli* total RNA, 75 pmol of 16S rRNA bridging nucleic acid or 75 pmol of 23S rRNA bridging nucleic acid with 75 μ l of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed	% 23S Removed
n.a./d23S-581	-	98.98529
n.a./d23S-581	-	98.87251
n.a./d23S-1118	-	93.62175
n.a./d23S-1118	-	91.4927
n.a./d23S-1954	-	98.68262
n.a./d23S-1954	-	99.03237
n.a./d23S-2511	-	99.31982
n.a./d23S-2511	-	99.13291
d16S-358/n.a.	97.65586	-
d16S-358/n.a.	97.51393	
d16S-537/n.a.	99.16427	-
d16S-537/n.a.	98.92345	-
d16S-807/n.a.	98.0661	•
d16S-807/n.a.	98.14292	-

n.a. = not applicable

The following results are from reactions that employed 5 μ g of *E. coli* total RNA, 25 pmol of each 16S rRNA or 23S rRNA bridging nucleic acid, and 25 μ l of capture nucleic acid described in Example 1. The rRNA/bridging nucleic acid annealing reaction was for 60 minutes at 37°C.

Bridging Nucleic Acid 168/238	% 16S Removed	% 23S Removed
n.a./d23S-488	-	~100
n.a./d23S-1118	-	~100
d16S-3'/d23S-488	89.024	94.228
d16S-548/d23S-488	~100	93.718
d16S-1092/d23S-488	~100	92.652

The following results are from reactions that employed 5 µg of E. coli total RNA, 16S

10 rRNA bridging nucleic acid as indicated, 23S rRNA bridging nucleic acid as indicated, and 25 µl

of capture nucleic acid described in Example 1. The rRNA/bridging nucleic acid annealing
reaction was for 120 minutes at 37°C.

Bridging Nucleic Acid		
16S/23S	% 16S Removed	% 23S Removed
d16S-3' (25 pmol)/n.a.	89.137	-
d16S-548 (25 pmol)/n.a.	~100	
d16S-1092 (25 pmol)/n.a.	~100	-
d16S-3' (25 pmol)		
d16S-548 (25 pmol)/n.a.	~100	
d16S-3' (25 pmol)		
d16S-1092 (25 pmol)/n.a.	~100	
d16S-548 (25 pmol)		
d16S-1092 (25 pmol)n.a.	~100	-
d16S-548 (25 pmol)/		
d23S-3' (25 pmol)	~100	~100
d16S-1092 (25 pmol)/		
d23S-3' (25 pmol)	~100	~100
d16S-3' (25 pmol)/		
d23S-3' (25 pmol)	92	~100

EXAMPLE 4: The Effect of Washing the Capture Nucleic Acid

The purpose of this experiment was to determine if washing the capture nucleic acid and combining the wash with the purified mRNA had an effect on the presence of rRNA in the purified mRNA sample. Reactions employed $10~\mu g$ of E.~coli total RNA, 75 pmol d16S-1092, 75 pmol of d23S-d1118, and $100~\mu l$ of capture nucleic acid described in Example 1. The rRNA/bridging nucleic acid annealing reaction proceeded for 60 min at 37° C. After the nucleic acid capture step, the capture nucleic acid (with bound rRNA) was resuspended and washed with $100~\mu l$ of the indicated solution at room temperature for 5 minutes. The capture nucleic acid was re-captured with a magnetic stand and the supernatant was removed and combined with mRNA in the supernatant from the first capture. mRNA in the combined supernatants were precipitated with ethanol and evaluated with RNA 6000 Lab Chip assay for the presence of rRNAs. The percent of rRNA removal for the entire process is indicated in the table below.

Wash	% 16S Removed	% 23S Removed
0.4 M TMAC	66.061	66.175
1.0 M TMAC	95.810	96.708
1.5 M TMAC	~100	~100
2.0 M TMAC	~100	~100

15

20

25

10

These results demonstrate that lowering the molarity of the TMAC wash solution increases the stringency of the rRNA capture reaction when the temperature is held constant at room temperature. The results also demonstrate that washing the capture nucleic acid magnetic beads with 1.5 and 2.0 M TMAC does not remove rRNA from the capture nucleic acid.

EXAMPLE 5:

Evaluation of Efficacy with Prokaryotic and Eukaryotic rRNA Targets

The purpose of this example was to evaluate efficacy of the methods of the invention for depleting 16S rRNA, 18S rRNA, 23S rRNA, and 28S rRNA from mixtures of prokaryotic and eukaryotic total RNA. Depletion methods were verified using various mammalian samples, including rat livers.

Equal amounts (2.5 µg) of E. coli total RNA and rat liver total RNA were mixed prior to the mRNA enrichment procedure. The bridging oligonucleotides employed were:

	d16S-1092	(10 pmol)
	d16S-807	(10 pmol)
	d23S-1954	(10 pmol)
	d23S-2511	(10 pmol)
5	d18S-3711	(20 pmol)
	d28S-11599	(20 pmol)

The reaction used 50 µl of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to methods in Example 2. The results are shown in FIG. 5A and 5B. Note that all rRNAs were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

EXAMPLE 6:

Evaluation of Efficacy with Human rRNA Targets

Additional experiments were done using human samples to evaluate the extent of human rRNA depletion using the bridging oligonucleotides shown below. Depletion of 18S rRNA and 28S rRNA was observed from human liver total RNA. rRNAs were depleted from human liver total RNA (5 µg). The bridging oligonucleotides employed were:

d18S-3711 (40 pmol) d28S-11599 (40 pmol)

20

25

30

15

10

The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to Example 2.

The results are shown in FIG. 6A and 6B. Note that all rRNAs (18S, 28S) were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

EXAMPLE 7:

Evaluation of Efficacy with Rat rRNA Targets

Additional experiments were done using rat samples to evaluate the extent of rat rRNA depletion using the bridging oligonucleotides shown below. Depletion of 18S rRNA and 28S rRNA was observed from rat liver total RNA. rRNAs were depleted from rat liver total RNA (5 µg). The bridging oligonucleotides employed were:

d18S-3711R-polyA (40 pmol) d28S-11599R-polyA (40 pmol) The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to Example 2.

The results are shown in FIG. 7A and 7B. Note that all rRNAs (18S, 28S) were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

EXAMPLE 8:

Evaluation of Efficacy with Mouse rRNA Targets

Additional experiments were done using mouse samples to evaluate the extent of rat rRNA depletion using the bridging oligonucleotides shown below. Depletion of 18S rRNA and 28S rRNA was observed from mouse liver total RNA (5 μ g). The bridging oligonucleotides employed were:

d18S-3711R-polyA (40 pmol)

5

20

25

d28S-11599R-polyA (40 pmol)

The reaction used 50 μ l of capture nucleic acid as described in Example 1. No wash step was employed. Otherwise the reaction was performed according to Example 2.

The results are shown in FIG. 8A and 8B. Note that all rRNAs (18S, 28S) were depleted except the 5S and 5.8S rRNAs for which no bridging oligonucleotides were added.

EXAMPLE 9:

Use of Purified E. coli mRNA in Gene Array Expression Analysis

mRNA was purified from total E. coli RNA (10 μ g) using the methods of the invention as described in Example 2. A control reaction was also performed in which the bridging nucleic acid mixture was omitted form the reaction. Control total RNA and purified mRNA (1.5 μ g) were added to 70 pmol random hexamers in a final volume of 7.25 μ l. The mixture was heated at 70° C for 10 minutes, then transferred to ice for 3 minutes. The following components were added to each reaction:

5 μl cDNA 1st strand synthesis buffer (Invitrogen)

2.5 μl 0.1 M DTT

1.25 µl 10 mM dATP

WO 03/054162 PCT/US02/41014

67

1 μΙ	Superscript II reverse transcriptase (Invitrogen) 200 U/µI	
5 μ1	10 mCi/ml	³³ P-dCTP (Perkin Elmer-NEN)
1.25 µl	10 mM dTTP	
1.25 pt	10 11111 4011	

10 mM dGTP

1 25 11

5

10

15

The reactions were incubated at 42°C for 120 minutes. Unincorporated nucleotides were removed from the reactions with a Qiaquick PCR cleanup column (Qiagen). The labeled cDNAs (3 x 10⁷ cpm/blot) were used to probe replicate portions of PanoramaTM E. coli gene arrays, using hybridization buffers supplied by the array manufacturer (Sigma-Genosys). The arrays were washed and exposed to film. This example demonstrates a dramatic increase in hybridization signal (sensitivity) on gene arrays when labeled cDNA is prepared from bacterial mRNA, purified according to the methods of the invention, rather than from total RNA.

EXAMPLE 10:

Instructions for Use with Kit

The following instructions have been followed with a kit of the invention described below for the successful depletion of 16S and 23S rRNA from a sample comprising prokaryotic RNA populations. Bridging oligonucleotides with targeting regions complementary to 18S and 28S rRNA may be employed according to the method below to effect a similar result (as in Examples 5-8).

20 Materials Provided with a Kit Embodiment

	30 μ1	Control RNA
	1.2 ml	Capture Nucleic Acid [as in Example 1]
	7 ml	Binding Buffer [as in Example 1]
	95 μl	Bridging Oligonucleotide Mix [as in Example 2]
25	2.4 ml	Wash Solution [as in Example 1]
	1.75 ml	Nuclease-free Water
	50 ea	RNase-free 1.5 ml tubes
	25 ea	RNase-free 2ml Elution tubes
	200 μ1	Glycogen (5 mg/ml)
30	875 µl	3 M NaOAc

PCT/US02/41014

Experimental Parameters

10

15

20

25

30

A. RNA Source

This mRNA enrichment procedure is designed to work with purified total RNA from many different bacteria, including both gram-positive and gram-negative species. The procedure was optimized with total E. coli RNA and has been found to remove 90-99% of the rRNA from Bacillus subtilis, Staphylococcus aureus, Prochlorococcus sp., Neisseria meningitidis, and Pseudomonas aeruginosa, for example. It is contemplated that any eubacterial species may be targeted using the methods and compositions of the invention.

68

This procedure is designed so that small RNAs (including tRNA and 5S rRNA) remain in the enriched mRNA population. However, if the loss of very small RNA species (<200 base) will not be an issue, the initial isolation of total RNA should be performed with Ambion's RNAQUEOUS KIT. The RNAQUEOUS KIT will remove most small RNA species and provide the highest possible level of mRNA enrichment. If small RNAs are of interest to the user, it is best to avoid glass fiber filter-based purification.

B. Precipitate RNA to remove salt and concentrate if necessary

Total RNA prepared from a solid-phase extraction method such as RNAQUEOUS can be used immediately after elution because such samples are unlikely to have high levels of salt. On the other hand, RNA isolated by methods that include organic extractions, for example using the products RNAWIZ, TRIZOL or TOTALLY RNA, may have a substantial amount of residual salt. If RNA from these types of procedures has been precipitated only a single time, we recommend doing a second alcohol precipitation and 70% EtOH wash to remove residual salt before starting the enrichment procedure.

The recommended maximum amount of RNA per reaction is 10 μ g and the recommended maximum volume for the RNA is 15 μ l. If the RNA sample is too dilute, it will be necessary to precipitate and concentrate the RNA to at least 10 μ g/15 μ l. Precipitate the RNA with:

 ^{0.1} volume 5 M Ammonium Acetate or 3 M sodium acetate

^{• 1} µl Glycogen (The glycogen acts as a carrier to increase precipitation efficiency from dilute RNA solutions; it is unnecessary for solutions with 200 µg RNA/ml)

 ^{2.5} volumes 100% ethanol

- Leave the precipitation mixture at -20°C overnight, or quick-freeze it in either ethanol and dry ice. or in a -70°C freezer for 30 minutes.
 - b. Recover the RNA by centrifugation at 12,000 x g for 30 minutes at 4°C.
- 5 c. Carefully remove and discard the supernatant. The RNA pellet may not adhere tightly to the walls of the tubes, so we suggest removing the supernatant by gentle aspiration with a fine-tipped pipette.
 - d. Centrifuge the tube briefly a second time, and aspirate any additional fluid that collects with a fine-tipped pipette.
- 10 e. Add 1 ml 70% ethanol, and vortex the tube a few times. Repellet the RNA by microcentrifuging, for 10 minutes at 4°C. Remove supernatant carefully as in steps c and d above.

RNA should be dissolved in TE or Ambion's THE RNA STORAGE SOLUTION. It is important to accurately quantitate RNA so as not to overload the system. Ambion recommends using the RiboGreen RNA Quantitation Assay and Kit (Molecular Probes) or a high quality, calibrated spectrophotometer.

C. Save an aliquot of your total RNA

If possible, retain a small aliquot (~1-2 µg) of the total RNA used for comparison with enriched mRNA by gel electrophoresis after the procedure is finished.

20 Instructions

25

Anneal RNA and Bridging Oligonucleotide Mix

1. Add RNA to Binding buffer

Add total RNA (up to 10 µg total RNA in a maximum volume of 15 µl) to 200 µl Binding Buffer in a 1.5 ml tube provided with the kit. Close the tube and tap or vortex gently to mix.

Add Bridging Oligonucleotide Mix to RNA

Add 4.0 μ l of the Bridging Oligonucleotide Mix to the RNA in Binding Buffer. Close the tube and tap or vortex gently to mix. Pulse in a microcentrifuge very briefly to get mixture to bottom of tube.

Incubate reactions at 70°C for 10 minutes.

Incubating the mixture at 70°C for 10 minutes denatures secondary structures in RNA, including the 16S and 23S rRNAs, allowing for maximal hybridization of the bridging oligonucleotides to the rRNAs.

Incubate reactions at 37°C for 1 hour.

Incubating the mixture at 37°C for 1 hour allows for binding of the bridging oligonucleotides to the 16S and 23S rRNA. The Binding Buffer has been optimized to function specifically and efficiently at this temperature.

B. Prepare the Capture Nucleic Acid

10

During the 1 hour RNA/^{Bridging} Oligonucleotide Mix annealing step, prepare the Capture Nucleic Acid. The Capture Nucleic Acid is in a 1% (10 mg/ml) suspension, vortex the tube briefly before pipetting to be sure they are well suspended.

1. Aliquot the Capture Nucleic Acid

For each 10 μ g reaction remove 50 μ l Capture Oligos to a 1.5 ml tube. Capture Nucleic 15 Acid for up to 10 reactions can be processed in a single 1.5 ml tube.

2. Wash the Capture Nucleic Acid once with water and once with Binding Buffer

- a. Capture the beads (Capture Nucleic Acid) by placing the tube on the Magnetic 20 Stand. Leave the tube on the stand until all of the Capture Nucleic Acid is arranged inside the tube near the magnet. This will take ~3 minutes for microfuge tubes.
 - Carefully remove the supernatant by aspiration, leaving the beads in the tube, and discard the supernatant.
 - Add Nuclease Free Water to the captured beads at a ratio of 50 μl/50 μl beads).
- 25 d. Remove the tube from the Magnetic Stand, resuspend the beads by gently vortexing briefly, recapture the beads with a Magnetic Stand, carefully aspirate the supernatant, leaving the beads in the tube, and discard the supernatant.
 - e. Add Binding Buffer to the captured beads at a ratio of 50 μl/50 μl beads).

71

f. Repeat step d.

10

20

- 3. Resuspend the Capture Nucleic Acid in Binding Buffer
- Add Binding Buffer to the captured beads at a ratio of 50 μl/50 μl beads).
- Remove the tube from the Magnetic Stand, resuspend the beads by gently tapping
 the tube or very gentle vortexing.
 - Pulse spin in a microcentrifuge to get liquid to the bottom of the tube.
 - C. Capture the rRNA with Capture Nucleic Acid and Recover the Enriched mRNA
 - Add Capture Nucleic Acid (50 µl/rxn) to RNA/Bridging Oligonucleotide Mix and incubate at RT for 15 minutes.
 - a After the 1 hour incubation at 37°C (Step A.4) remove tubes to room temperature (RT) and immediately add 50 μ l of the washed and equilibrated beads (Capture Nucleic Acid, from Step B.3c) to each purification reaction. Very gently vortex or tap tube to mix briefly and pulse spin in a microcentrifuge to get liquid to the bottom of the tube.
- b. Incubate 15 minutes at RT. During this step the oligonucleotide sequence on the Capture Nucleic Acid anneals to the bridging oligonucleotides. The bridging oligonucleotides remain hybridized to the 16S and 23S rRNAs. The hybridization "sandwich" of bridging oligonucleotide and capture oligonucleotide (via the capture region on the capture oligo and the bridging region on the bridging oligo) is formed at this step.

Recover the supernatant containing the enriched mRNA.

- a. Capture the beads by placing the tube on the Magnetic Stand. Leave the tube on the stand until all of the beads are arranged inside the tube near the magnet. This will take ~3 minutes for microfuge tubes. Allow the beads to be completely captured by the magnet for at least 3 minutes
- 25 b. Remove the supernatant by aspiration, being careful not to dislodge the beads. Put the supernatant into a 2 ml nipple bottom tube on ice and save. Do not be overly concerned if there seems to be beads in the removed supernatant. The excess can be removed at the end of the procedure. The supernatant contains the enriched mRNA sample.

- 3. Wash the Oligo MagBeads with Wash Solution and recover the wash.
- a. Add Wash Solution to the captured beads at a ratio of 100 μ l Wash Solution/50 μ l beads.
- Remove the tube from the Magnetic Stand, resuspend the beads by gently
 vortexing briefly.
 - c. Incubate at RT for 5 minutes.
 - d. Recapture the beads with the Magnetic Stand as in step C.2a. Allow the beads to be completely captured by the magnet for at least 3 minutes.
- e. Remove the supernatant by aspiration, being careful not to dislodge the beads.

 10 Put this supernatant in the 2 ml nipple bottom tube on ice with that from step C.2b.
 - D. Precipitate and resuspend the enriched mRNA in the supernatant.
 - 1. Perform an EtOH precipitation on the collected supernatant.
- a. Add 1/10 Volume 3M NaOAc (35 µl) and 5 mg/ml glycogen to a final 15 concentration of 100µg/ml (7 µl) to the supernatant from step C.3.e. (the supernatant volume should be ~350 µl).
 - b. Briefly vortex the sample to mix.
 - Add 3 Vol. ice cold 100% EtOH (1175 μl) and mix well by vortexing the sample.
 - d. Precipitate the sample at -20°C for at least 1 hour.
 - e. Centrifuge the sample for 30 min. @ 13,000 rpm.
 - f. Carefully decant the supernatant.
 - g. Add 750ml ice cold 70% EtOH, vortex briefly, and centrifuge for 5 min. @ 13,000 rpm. Decant the supernatant.
 - h. Repeat step D.1.g.

PCT/US02/41014

73

 After decanting the supernatant spin briefly to collect. Remove the remaining supernatant with a pipettor, being careful not to dislodge the pellet. Air dry for 5 min.

2. Resuspend the enriched mRNA in an appropriate buffer.

- a. After the pellet has air dried for no more than 5 min. add 2 μl TE pH 8.0 (RNA STORAGE SOLUTION. 1 mM EDTA or Nuclease-Free ddH-O could be substituted).
 - b. Allow the RNA to resuspend for 15 min. at room temperature. Vortex the sample vigorously to resuspend. Collect the sample by brief centrifugation. NOTE: If the pellet refuses to go into solution the sample can be incubated for 5 min. @ 70°C. This should help resuspend the pellet. NOTE: Often there will be beads remaining in the sample after the precipitation (This will cause the RNA solution to appear brownish in color). This can be remedied by applying the sample to the Magnetic stand for ~3 min. and removing the supernatant to a new tube.

E. Compatibility with respect to other microorganisms

Based on experimental evidence and sequence information, the following organisms should be compatible (removal of 16S rRNA and of 23S rRNA) with the oligos identified in 15 Example 1 (non-control oligos): Acidithiobacillus ferrooxidans, Acinetobacter calcoaceticus, Actinobacillus actinomycetemcomitans, Aeromonas hydrophila, Agrobacterium tumefaciens, Alcaligenes faecalis, Bacillus alcalophilus, Bacillus anthracis, Bacillus cereus, Bacillus halodurans, Bacillus licheniformis, Bacillus mycoides, Bacillus subtilis, Bacillus thuringiensis, 20 Bartonella bacilliformis, Bordetella avium, Bordetella bronchiseptica, Bordetella parapertussis, Bordetella pertussis, Borrelia burgdorferi, Bradyrhizobium japonicum, Bradyrhizobium lupini, Brevundimonas diminunata, Brucella melitensis, Brucella melitensis biovar suis, Buchnera aphidicola, Buchnera sp. APS, Burkholderia cepacia, Burkholderia mallei, Burkholderia pseudomallei. Caulobacter crescentus, Chlamydia muridarum, Chlamydia suis, Chlamydia 25 trachomatis. Chlamydophila abortus. Chlamydophila caviae, Chlamydophila felis, Chlamydophila pecorum, Chlamydophila pneumoniae, Chlamydophila psittaci, Chlorobium limicola, Chlorobium tepidum, Citrobacter freundii, Clostridium acetobutylicum, Clostridium difficile. Clostridium histolyticum, Corynebacterium diptheriae, Corynebacterium glutamicum, Cytophaga hutchisonii, Desulfovibrio vulgaris, Dichelobacter nodosus, Enterococcus asini, 30 Enterococcus avium, Enterococcus casseliflavus, Enterococcus cecorum, Enterococcus

columbae. Enterococcus dispar, Enterococcus durans. Enterococcus faecalis, Enterococcus faecium, Enterococcus flavescens, Enterococcus gallinarum, Enterococcus hirae, Enterococcus malodoratus, Enterococcus mundtii, Enterococcus pseudoavium, Enterococcus raffinosus, Enterococcus saccharolyticus, Enterococcus sulfureus, Erwinia chrysanthemi, Escherichia coli, Fibrobacter succinogenes, Frankia sp., Fusobacterium nucleatum. 5 stearothermonhilus. Geobacter sulfurreducens. Gluconacetobacter europaeus. Gluconacetobacter intermedius, Gluconacetobacter xylinus, Haemophilus ducreyi, Haemophilus influenzae, Klebsiella pneumoniae, Lactobacillus amylolyticus, Lactobacillus delbrueckii, Lactococcus lactis. Leuconostoc carnosum. Leuconostoc lactis, Leuconostoc mesenteroides, Listeria gravi, Listeria innocua, Listeria ivanovii, Listeria monocytogenes, Listeria seeligeri, 10 Melissococcus plutonius, Micrococcus luteus, Mycobacterium avium, Mycobacterium avium supsp. Paratuberculosis, Mycobacterium bovis, Mycobacterium kansasii, Mycobacterium leprae, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis, Myxococcus xanthus, Neisseria gonorrhoeae, Neisseria meningitidis, Nitrosomonas europaea, Pasteurella multocida, Peptococcus niger, Plesiomonas shigelloides, Pseudomonas aeruginosa, 15 Pseudomonas putida. Pseudomonas syringae, Ralstonia pickettii, Ralstonia solanacearum, Renibacterium salmoninarum, Rhizobium vitis, Rhodococcus erythropolis, Rhodococcus fascians. Rhodopseudomonas palustris. Rhodospirillum rubrum, Rickettsia akari, Rickettsia australis. Rickettsia bellii, Rickettsia canadensis, Rickettsia conorii, Rickettsia montanensis. 20 Rickettsia parkeri, Rickettsia prowazekii, Rickettsia rhipicephali, Rickettsia rickettsii, Rickettsia sibirica. Rickettsia typhi, Salmonella bongori, Salmonella enterica, Salmonella enteritidis, Salmonella paratyphi, Salmonella typhi, Salmonella typhimurium, Shewanella putrefaciens, Sinorhizobium meliloti. Sporosarcina globispora, Staphylococcus aureus, Staphylococcus carnosus, Staphylococcus condimenti, Staphylococcus epidermidis, Stigmatella aurantiaca, Streptococcus equii, Streptococcus gordonii, Streptococcus macedonicus, Streptococcus mitis, 25 Streptococcus mutans, Streptococcus oralis, Streptococcus parauberis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus thermophilus, Streptococcus uberis, Streptomyces ambofaciens, Streptomyces coelicolor, Streptomyces griseus, Streptomyces lividans. Streptomyces nodosus, Streptomyces rimosus, Thermoanaerobacter tengcongensis, Thermohifida fusca. Thermomonospora chromogena. Thiobacillus ferrooxidans, Trteponema 30 denticola, Treponema pallidum, Vibrio cholerae, Yersinia enterocolitica, Yersinia pestis, Xanthomonas campestris, Xanthomonas axonopodis pv. Citri, and Xylella fastidiosa.

Based on experimental evidence and sequence information, the following organisms should be partially compatible (removal of 23S rRNA and of 50-100% 16S rRNA) with oligos identified in Example 1 (non-control): Azotobacter vinelandii, Bacteroides fragilis, Carboxydothermus hydrogenoformans, Clostridium tyrobutyricum, Desulfovibrio vulgaris, Dictyoglomus thermophilum, Enterococcus solitarius, Erysipelothrix rhusiopathiae, Erysipelothrix tonsillarum, Flexibacter flexilis, Legionella pneumophila, Leptospira interrogans, Leucothrix mucor, Listeria welshimeri, Methylococcus capsulatus, Myroides odoratus, Oenococcus oeni, Paracoccus denitrificans, Pectinatus frisingensis, Porphyromonas gingivalis, Prevotella intermedia, Silicibacter pomeroyi, Tannerella forsythensis, Tetragenococcus halophilus, Thermobispora bispora, Thermus thermophilus and, Thiomonas cuprina

Based on experimental evidence and sequence information, the following organisms should be partially compatible (removal of 16S rRNA and 50-100% of 23S rRNA) with oligos identified in Example 1 (non-control): Burkholderia fungorum, Clostridium perfringens, Desulfitobacterium hafniense, Magnetospirillum magnetotacticum, Mesorhizobium loti, Nannocystis exedens, Novosphingobium aromaticivorans, Parachlamydia acanthamoebae, Ruminobacter amylophilus, Ruminococcus albus, Tropheryma whipplei, and Wolbachia endosymbiont of Drosophila melanogaster.

Based on experimental data and sequence information, the following organisms are believed to be incompatible with the oligos in Example 1: Archaebacteria, Campylobacter spp., Chloroflexus aurantiacus, Cyanobacteria, Dehalococcoides ethenogenes, Deinococcus radiodurans, Fervidobacterium islandicum, Helicobacter pylori, Mycoplasma spp., Pirellula marina, Propionibacterium freundenreichii, Simkania negevensis, Thermotoga maritima, and Ureaplasma urealyticum.

EXAMPLE 11: <u>Methods for Eukaryotic rRNA Depletion from Mixed Human/E. coli</u> Total RNA

These experiments were performed to demonstrate the depletion of 18S and 28S rRNAs from a mixture of prokaryotic and eukaryotic total RNA. Materials from Example 1 were used in the following experiments, except where noted.

30 RNA/Bridging Nucleic Acid Mixture Annealing

20

RNA (50 µg human total / 0.5 µg *E. coli* total) in 30 µl TE pH8.0 was added to 300 µl of binding buffer (The binding buffer in this example contains 0.02% Triton-X 100 which has been shown to reduce non-specific interactions between nucleic acids target and the capture nucleic acid). The bridging nucleic acid mixture consisted of d18S-3711, d18S-4238, d18S-5482, d28S-7979, d28S-11599 and d28S-12533 (each of these bridging oligonucleotides is at a final concentration of 3.33 µM). The bridging nucleic acid mixture (20 µl) was added to the RNA and the mixture was incubated at 70°C for 10 minutes and then shifted to 37°C for 1 hour.

Preparation of Capture Nucleic Acid

Capture nucleic acid (Oligo (dT) MagBeads, Seradyn) in storage buffer was mixed and 250 µl was removed to a separate tube. A magnetic stand was applied to the side of the tube to capture the magnetic beads and the supernatant was removed. The capture nucleic acid was equilibrated one time with distilled, deionized water (250 µl) and once with binding buffer (250 µl). The captured nucleic acid was captured again with a magnetic stand, and the binding buffer wash was removed. The magnetic beads were stored on ice until used in the next step (rRNA Capture).

rRNA Capture

10

15

20

25

30

Following the 1 hour annealing of RNA with the bridging nucleic acid mixture, the RNAbridging oligonucleotide mixture was added to the capture nucleic acid (see Preparation of Capture Nucleic Acid), and the mixture was incubated at 37°C for 15 minutes. A magnetic stand was applied to the tube to capture the magnetic beads. The supernatant containing E. coli total RNA was removed to another tube and saved. An optional washing step was performed. The magnetic beads were washed with Wash Solution (100 µI) and captured again. The wash supernatant was removed and added to the original supernatant.

Fifteen minutes was found to be an adequate length of time for rRNA capture. Longer time periods can be used with no adverse effects. rRNA capture occurs rapidly, and capture times of 5 - 60 minutes have been used successfully in the methods of the invention.

Precipitating RNA

E. coli total RNA was precipitated by adding 1/10 volume of 3 M NaOAc (pH 5.5) and 3 volumes of 100% EtOH and incubating at -20°C for 60 minutes. The precipitated RNA was pelleted in a microfuge, washed with 70% EtOH, and resuspended in TE (pH 8.0).

77

Analysis of Purified RNA

Purified RNA was analyzed with the Caliper RNA 6000 LabChip kit on an Agilent Bioanalyzer. Purified RNA was compared with a control total RNA sample that was carried through the reaction as described above, except that the Bridging Nucleic Acid Mixture was omitted (FIG. 9). The percentage of a rRNA present in the sample is calculated from the area under the peak. The percentage removal of the 18S and 28S rRNAs was calculated as described in Example 2 for removal of 16S and 23S rRNAs.

Electropherograms of RNA from a control reaction and from an experimental reaction after ribosomal RNA depletion are shown in FIG. 9.

10

15

20

25

EXAMPLE 12:

Evaluation of efficacy with Mixed Prokaryotic and Eukaryotic rRNA Targets

The purpose of this experiment was to determine if one could, first remove the eukaryotic RNA and subsequently remove the prokaryotic rRNAs from a mixture of the two total RNAs. The materials and methods of Examples 1, 2 and 11 were used, except where noted. Depletion methods were verified using various mammalian samples, including rat liver total RNA, and both E. coli and Bacillus subtilits total RNA.

25 μg rat liver total RNA and 2 μg E. coli total RNA were mixed prior to the RNA enrichment procedure. The bridging oligonucleotides employed were: d16S-807, d16S-1092, d23S-1954, d23S-2511, d18S-3711, d18S-4238, d18S-5482, d28S-7979, d28S-11599, and d28S-12533.

The reactions began with procedures similar to Example 11, except for the following changes. 10 µl bridging nucleic acid mixture and 125 µl capture nucleic acid were used to remove the mammalian 18S and 28S rRNAs. Following the wash step, the wash solution and the unbound fraction containing the bacterial RNA were combined. The precipitation step was not performed. Instead the bacterial 16S and 23S rRNA was removed as in Example 2, with the following modifications. The bridging nucleic acid mixture (4 µl) containing d16S-807, d16S-1092, d23S-1954, and d23S-2511 was added directly to the combined wash and unbound

fractions containing the bacterial RNA. The remainder of the procedure for 16S and 23S rRNA followed the methods from Example 2.

Electropherograms of RNA from a control reaction with no bridging nucleic acids (FIG. 10A), from a reaction following 18S and 28S rRNA removal (FIG. 10B), and from a reaction following subsequent removal of 16S and 23S rRNA (FIG. 10C) are shown in FIG. 10.

EXAMPLE 13:

<u>Use of Purified E. coli mRNA from Mixed Eukaryotic/Prokaryotic Samples in Gene Array</u> Expression Analysis

mRNA was purified from total *E. coli* RNA (2 μg) in a background of human total RNA

10 (25 μg) using the methods of the invention as described in Example 12 (16S, 23S, 18S and 28S

rRNAs were all depleted from the sample). A control reaction was also performed in which the

bridging nucleic acid mixtures were omitted from the reaction. Control total RNA (8.4 μg) and

purified mRNA (1.0 μg) were added to 160 pmol random decamers in a final volume of 24.5 μl.

These RNA amounts represent equal fractions of the control and purified RNA samples after the

procedure is complete. The mixture was heated at 70°C for 10 minutes, then transferred to ice

for 3 minutes. The following components were added to each reaction:

	12 µl	cDNA 1st strand synthesis buffer (Invitrogen)
	6 μl	0.1 M DTT
	3 μl	10 mM dATP
20	3 μl	10 mM dGTP
	3 μl	10 mM dTTP
	5 µl	10 mCi/ml ³³ P-dCTP (Perkin Elmer-NEN)
	1 μl	RNase Inhibitor (cloned)

- 25 The reaction was then incubated at room temperature for 10 minutes, and the following component was added:
 - 2 ul Superscript II reverse transcriptase (Invitrogen) 200 U/ul

The reactions were incubated at 42°C for 120 minutes. Unincorporated nucleotides were removed from the reactions with a Qiaquick PCR™ cleanup column (Qiagen). RNA present in the cDNA probes was hydrolyzed by incubation at 65°C for 10 minutes in .05 N NaOH. The probes were subsequently neutralized with 0.05 M HCl. The labeled cDNAs (5 x 10⁷ cpm/blot) 5 were used to probe replicate portions of Panorama™ E. coli gene arrays, using hybridization buffers supplied by the array manufacturer (Sigma-Genosys). The arrays were washed and exposed to film. This example demonstrates a dramatic increase in hybridization signal (sensitivity) on gene arrays when labeled cDNA is prepared from enriched bacterial mRNA, purified according to the methods of the invention, rather than from the mixed prokaryotic and eukaryotic total RNA.

Example 14:

Evaluations of Efficacy with non-E.coli Prokaryotic Targets

The materials and methods of Examples 1 and 2 were employed in the Examples below except where noted. These experiments were performed to evaluate the efficacy of various bridging nucleic acids with different bacterial species.

Additional targeting regions for prokaryotic 16S and 23S rRNAs were designed. The targeting regions are shown, in the examples below, 3' of the bridging regions. Thus, the targeting region encompasses the remaining, non-bridging region of each molecule described below. SEO ID NOs are provided for the targeting regions of the bridging nucleic acids provided below (i.e., sequence of bridging regions not included in SEQ ID NO.). Furthermore, the oligos have been further designated with a suffix at the end of the oligo number. CY refers to cyanobacteria; P refers to pseudomonas; R refers to rhodobacter; and CH refers to campylobacter/helicobacter.

16S prokaryotic rRNA bridging oligonucleotides

25 d16S-1114P

5'-AAAAAAAAAAAAAAAAAGGGTTGCGCTCGTTACGGGACTT-3' (SEO ID NO:74)

d16S-1114R

5'-AAAAAAAAAAAAAAAAAGGGTTGCGCTCGTTGCCGGACTT-3' (SEQ ID NO:75)

d16S-364

10

15

20

30

5'-AAAAAAAAAAAAAAAAAATCCCCACTGCTGCCTCCCGTAGG-3' (SEQ ID NO:76)

d16S-1087

35

d23S-518 CH

- d16S-364CY 5'-AAAAAAAAAAAAAAAAATCCCCACTGCTCCCGTAGG-3' (SEO ID NO:78) d16S-534CY 5'-AAAAAAAAAAAAAAAAAATTACCGCGGCTGCTGGCACGGA-3' (SEO ID NO:79) 416S-928CY 10 5'-AAAAAAAAAAAAAAAAAACCCCGTCAATTCCTTTGAGTTTC-3' (SEQ ID NO:80) d16S-1087CY 23S prokaryotic rRNA bridging oligonucleotides d23S-479RCH 5'-AAAAAAAAAAAAAAAAATTTCACCTTTCCCTCACGGTACT-3' (SEO ID NO:82) 20 d23S-485 5'-AAAAAAAAAAAAAAAAAGGTTCTTTTCACCTTTCCCTCGC-3' (SEO ID NO:83)
- 5'-AAAAAAAAAAAAAAAAAAAAATGGTTTCAGGTTCTATTTCACTC-3' (SEQ ID NO:84) 25 d23S-1954 CH
- 5'-AAAAAAAAAAAAAAAAAAAATTTAACCGACAAGGAATTTCGC-3' (SEQ ID NO:85) d23S-485CY 30 5'-AAAAAAAAAAAAAAAAAAGGTTCTTTTCACCTTTCCCTCGC-3' (SEQ ID NO:86)
 - The following results are from reactions that employed 10 ug of Pseudomonas

aeruginosa total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-1954 (20 pmol), d23S-2511 (20 pmol)	90.1%	99.5%
d16S-807 (20 pmol), d16S-1114P (20 pmol) d23S-1954 (20 pmol), d23S-2511 (20 pmol)	97.0%	99.4%

The following results are from reactions that employed 10 µg of Bacillus subtilis total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1.

Bridging Nucleic Acid 16S/23S	% 16S Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-1954 (20 pmol), d23S-2511 (20 pmol)	98.2%	96.2%

5

The following results are from reactions that employed 10 µg of Campylobacter fetus total RNA, 40 pmol of 16S bridging nucleic acid and 40 pmol of 23S bridging nucleic acid, and 50 µl of capture nucleic acid described in Example 1. The Campylobacter fetus 23S rRNA is processed into two fragments (FIG. 12).

Bridging Nucleic Acld 16S/23S	% 16S Removed average of 2 reactions	% 23S (1260 nt fragment) Removed average of 2 reactions	% 23S (1667 nt fragment) Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	97.3%	97.7%	89.3%
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-518CH (20 pmol), d23S-2511	95.9%	96.7%	89.5%

10

The following results are from reactions that employed 10 µg of Rhodobacter sphaeroides total RNA, 40 pmol of 16S bridging nucleic acid, and 40 pmol of 23S bridging nucleic acid, and 50 µl of capture nucleic acid, as described in Example 1 unless otherwise noted. The Rhodobacter sphaeroides 23S rRNA is processed into two fragments (FIG. 13). One fragment migrates with the 16S rRNA.

Bridging Nucleic Acid 165/235	% 16S + 23S fragment (1600 nt) Removed average of 2 reactions	% 23S Removed average of 2 reactions
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	81.6%	96.8%
d16S-807 (20 pmol), d16S-1092 (20 pmol) d23S-518CH (20 pmol), d23S-2511 (20 pmol)	95.9%	96.7%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	89.3%	96.1%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol), d23S-2511 (20 pmol)	97.0%	96.4%
d16S-807 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-2511 (20 pmol)	83.1%	96.5%
d16S-807 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	90.2%	95.9%
d16S-537 (20 pmol), d16S-807 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	96.7%	94.9%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	95.0%	90.2%
d16S-537 (20 pmol), d16S-1114R(20 pmol) d23S-479CH (20 pmol), d23S-1954 (20 pmol) d23S-2511 (20 pmol)	95.2%	89.8%

The results demonstrate that bridging nucleic acids will function with various species.

This example also demonstrates functionality based on sequence comparison, i.e., that a bridging oligonucleotide will function with rRNAs in different organisms based on sequence identity between the oligonucleotide and the rRNA of the organism.

Evaluations of Efficacy with Cyanobacteria Targets

10

The materials and methods of Examples 1 and 2 were employed except where noted. These experiments were performed to evaluate the efficacy of various bridging nucleic acids with different bacterial species.

The following results are from reactions that employed 10 µg of Anabaena spp. total RNA, the indicated amounts of the bridging nucleic acids, and 50 µl of capture nucleic acid described in Example 1. The Anabaena sp. 23S rRNA and 23S rRNAs from other cyanobacteria may be processed into several fragments (FIG. 14).

	% 16S Removed		23S Remov	
Bridging Nucleic Acld	average of	520 nt	2090 nt	2470 nt
16S/23S	2 reactions	fragment	fragment	fragment
d16S-364CY (20 pmol), d16S-	94.3	NA	NA	NA
928CY (20 pmol)	94.3	INA	INA.	I NA
d16S-364CY (20 pmol), d16S-	93.1	NA	NA	NA
1087CY (20 pmol)	93.1	INA	I NA	INA
d16S-928CY (20 pmol), d16S-	93.7	NA	NA	NA
1087CY (20 pmol)	93.7	NA.	NA.	I NA
d23S-485 (20 pmol), d23S-	NA	98.6	94.1	99.5
1954 (20 pmol)	NA NA	98.0	94.1	99.3
1934 (20 pmol)		00.4	05.7	99.5
d23S-485 (20 pmol), d23S-	NA	98.4	95.7	99.5
2511 (20 pmol) d16S-364CY (20 pmol), d16S-	00.5	96.5	84.7	97.7
	99.5	90.5	84.7	91.1
928CY (20 pmol) d23S-485, (20 pmol), d23S-				
1954 (20 pmol)	l			l
d16S-364CY (20 pmol), d16S-	99.2	96.3	87.2	98.5
928CY (20 pmol)	99.2	90.3	87.2	96.3
d23S-485, (20 pmol), d23S-				1
2511 (20 pmol)				1
d16S-364CY (20 pmol), d16S-	99.7	98.6	88.3	98.3
1087CY (20 pmol)	99.7	98.0	88.3	96.3
d23S-485 (20 pmol), d23S-		1		
1954 (20 pmol)		l		
d16S-364CY (20 pmol), d16S-	99.7	99.2	89.2	99.1
1087CY (20 pmol)	99.7	99.2	67.2	22.1
d23S-485 (20 pmol), d23S-				
2511 (20 pmol)				
d16S-928CY (20 pmol), d16S-	96.9	97.6	86.8	98.9
1087CY (20 pmol)	30.5	77.0	00.0	""
d23S-485 (20 pmol), d23S-				
2511 (20 pmol)				
d16S-364 (20 pmol), d16S-	99.2	98.2	88.1	97.7
1087CY (20 pmol)	, ,, <u>,,</u>	70.2	00.7	7
d23S-485 (20 pmol), d23S-				
1954 (20 pmol)	1			
d16S-364 (17.5 pmol), d16S-	99.8	98.8	88.4	98.5
1087CY (17.5 pmol)		,		
d23S-485 (20 pmol), d23S-				i
1954 (25 pmol)		l		l
d16S-364 (15 pmol), d16S-	99.8	99.1	90.6	98.3
1087CY (15 pmol)	1			
d23S-485 (20 pmol), d23S-				
1954 (30 pmol)	1			
d16S-364 (12.5 pmol), d16S-	99.9	98.7	92.5	98.9
1087CY (12.5 pmol)				
d23S-485 (20 pmol), d23S-	1			
1954 (35 pmol)			1.	l

	% 16S Removed		23S Remov age of 2 reac	
Bridging Nucleic Acid 16S/23S	average of 2 reactions	520 nt fragment	2090 nt fragment	2470 nt fragment
d16S-364 (10 pmol), d16S- 1087CY (10 pmol) d23S-485 (20 pmol), d23S- 1954 (40 pmol)	99.9	98.9	90.6	98.6

* * * * * * * * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents that are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

- 5 U.S. Application No. 09/854,412
 - U.S. Patent No. 4,486,539
 - U.S. Patent No. 4,563,419
 - U.S. Patent No. 4,659,774
- 10 U.S. Patent No. 4,682,195
 - U.S. Patent No. 4,683,202
 - U.S. Patent No. 4,751,177
 - U.S. Patent No. 4,816,571
 - U.S. Patent No. 4,868,105
- 15 U.S. Patent No. 4,894,325
 - U.S. Patent No. 4,959,463
 - U.S. Patent No. 5,124,246
 - U.S. Patent No. 5,141,813
 - U.S. Patent No. 5,200,314
- 20 U.S. Patent No. 5,214,136
 - U.S. Patent No. 5,216,141
 - U.S. Patent No. 5,223,618
 - U.S. Patent No. 5,264,566
 - U.S. Patent No. 5,273,882
- 25 U.S. Patent No. 5,288,609
 - U.S. Patent No. 5,378,825
 - U.S. Patent No. 5,412,087
 - U.S. Patent No. 5,428,148 U.S. Patent No. 5,432,272
- 30 U.S. Patent No. 5,445,934

 - U.S. Patent No. 5,446,137
 - U.S. Patent No. 5,457,025

T	T C	Dotont	NI.	5,466,786	
ŧ	1.5.	Patent	No.	3.400.780	

- U.S. Patent No. 5,470,967
- U.S. Patent No. 5,500,356
- U.S. Patent No. 5,539,082
- 5 U.S. Patent No. 5,554,744
 - U.S. Patent No. 5,574,146 U.S. Patent No. 5,589.335
 - U.S. Patent No. 5,602,240
 - U.S. Patent No. 5,602,244
- 10 U.S. Patent No. 5,610,289
 - U.S. Patent No. 5,614,617
 - U.S. Patent No. 5,623,070
 - U.S. Patent No. 5,645,897
 - U.S. Patent No. 5,652,099
- 15 U.S. Patent No. 5,670,663
 - U.S. Patent No. 5,672,697 U.S. Patent No. 5,681,947
 - U.S. Patent No. 5,700,922
 - U.S. Patent No. 5,702,896
- 20 U.S. Patent No. 5,708,154
 - U.S. Patent No. 5,709,629
 - U.S. Patent No. 5,714,324
 - U.S. Patent No. 5,714,331 U.S. Patent No. 5,714,606
- 25 U.S. Patent No. 5,719,262
 - U.S. Patent No. 5,723,597 U.S. Patent No. 5,736,336
 - U.S. Patent No. 5,744,305
 - U.S. Patent No. 5,759,777
- 30 U.S. Patent No. 5,763,167
 - U.S. Patent No. 5,766,855
 - U.S. Fatent 140. 3,700,03.
 - U.S. Patent No. 5,773,571

U.S. Patent No. 5	,777	,092
-------------------	------	------

- U.S. Patent No. 5,786,461
- U.S. Patent No. 5,792,847
- U.S. Patent No. 5,858,988
- 5 U.S. Patent No. 5,859,221
 - U.S. Patent No. 5,872,232
 - U.S. Patent No. 5,886,165 U.S. Patent No. 5,891,625
 - U.S. Patent No. 5,897,783
- 10 U.S. Patent No. 5,908,845
 - U.S. Patent No. 5,945,525
 - U.S. Patent No. 6,001,983
 - U.S. Patent No. 6,013,440
 - U.S. Patent No. 6,037,120
- 15 U.S. Patent No. 6,060,246 U.S. Patent No. 6,090,548
 - U.S. Patent No. 6,110,678
 - U.S. Patent No. 6,140,496
 - U.S. Patent No. 6,203,978
- 20 U.S. Patent No. 6,221,581
 - U.S. Patent No. 6,228,580
 - U.S. Patent No. 6,309,823
 - U.S. Patent No. 6.316,193 U.S. Patent No. 6,322,971
- 25 U.S. Patent No. 6,324,479
 - U.S. Patent No. 6,329,140
 - U.S. Patent No. 6,329,209
- 30 EP 266,032 PCT/EP/01219 PCT/US00/29865

WO 01/32672

WO 86/05815

WO90/06045

WO 92/20702

5

The entire issue of Current Opinion in Microbiology, Volume 4, February 2001.

Amara et al., Nucl. Acids Res. 25:3465-3470, 1997.

Arfin et al., J. Biol. Chem. 275:29672-29684.

10 Ausubel et al., In: Current Protocols in Molecular Biology, John, Wiley & Sons, Inc, New York, 1994.

Beaucage, Methods Mol. Biol. 20:33-61, 1993.

Chuang et al., J. Bacteriol. 175:2026-2036, 1993.

Coombes et al., Infect. Immun. 69:1420-1427, 2001.

15 Cornelis et al., Curr. Opin. Microbiol. 4:13-15, 2001.

Cummings et al., Emerg. Inf. Dis. 6:513-524, 2000.

DeRisi et al., Nature Genetics 14:457-460, 1996.

Detweller et al., Proc. Natl. Acad. Sci. USA 98:5850-5855, 2001.

Egholm et al., Nature 365(6446):566-568. 1993.

20 Feng et al., Proc. Natl. Acad. Sci. USA 97:6415-6420, 2000.

Fox, J.L. et al., ASM News 67:247-252, 2001.

Froehler et al., Nucleic Acids Res., 14(13):5399-5407, 1986.

Gillam et al., J. Biol. Chem. 253(8):2532-9, 1978.

Gillam et al., Gene 8(1):99-106, 1979.

25 Gingeras et al., ASM News 66:463-469, 2000.

Graham et al., Curr. Opin. Microbiol. 4:65-70, 2001.

Graham et al., Proc. Natl. Acad. Sci. USA 96:11554-11559, 1999.

Ichikawa et al., Proc. Natl. Acad. Sci. USA 97:9659-9664, 2000.

Itakura et al., J. Am. Chem. Soc. 97(25):7327-32, 1975.

30 Kagnoff et al., Curr. Opin. Microbiol. 4:246-250, 2001.

Khorana, Science 203(4381):614-25, 1979.

Klug et al., Methods Enzymol, 152:316-325, 1987.

89

Koshkin et al., Tetrahedron 54:3607-3630, 1998.

Koshkin et al., J. Am. Chem. Soc. 120:13252-13253, 1998.

Kricka, Nonisotopic DNA Probe Techniques, Academic Press, San Diego, California, 1992.

Liang et al., Methods Enzymol. 254:304-321, 1995.

5 Lockhart et al., Nature Biotech, 14:1675, 1996.

Maskos et al., Nuc. Acids. Res. 20:1679-1684, 1992.

Neidhardt et al., in Escherichia coli and Salmonella (Neidhardt, FC, Ed.), Vol. 1, pp.13-16, ASM Press, Washington, DC, 1996.

Newton et al., J Comput. Biol. 8:37-52, 2001.

10 Pietu et al., Genome Res. 6:492, 1996.

Plum, et al., Infect. Immun. 62:476-483, 1994.

Rappuoli, R. Proc. Natl. Acad. Sci. USA 97:13467-13469, 2000.

Robinson et al., Gene 148:137-141, 1994.

Rosenberger et al., J. Immunol. 164:5894-5904, 2000.

15 Sambrook et. al., In: Molecular Cloning: A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

Sambrook et al., In: Molecular Cloning: A Laboratory Manual, 3rd Ed., Cold Spring Harbor Press, Cold Spring Harbor, NY, 2001.

Schena et al., Science 270:467-470, 1995a.

20 Schena et al., Proc. Natl. Acad. Sci. USA 93:10539-11286, 1995b.

Shalon et al., Genome Res. 6:639-645, 1996.

Su et al., Molec, Biotechnol, 10:83-85, 1998.

Velculescu et al., Science 270:484-487, 1995.

Wahlestedt et al., PNAS 97:5633-5638, 2000.

25 Wei et al., J. Bacteriol. 183:545-556, 2001.

Wendisch, et al., Anal. Biochem. 290:205-213, 2001.

Wood et al., Proc. Natl. Acad. Sci. USA. 82:1585-1588, 1985.

Yoshida et al., Nucl. Acids Res. 29:683-692, 2001.

Zhao et al., Gene 156:207, 1995.

10

CLAIMS

- 1. A method for depleting or isolating a targeted nucleic acid from a sample comprising:
 - incubating the sample with a first bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the first targeting region and the targeted nucleic acid;
 - incubating the first bridging oligonucleotide with a capture oligonucleotide comprising a nonreacting structure and a capture region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the bridging region and the capture region; and
 - c) isolating the targeted nucleic acid from the remainder of the sample.
- 15 2. The method of claim 1 wherein the targeted nucleic acid is rRNA.
 - The method of claim 2, wherein the rRNA is prokaryotic 16S, prokaryotic 23S, eukaryotic 17S or 18S, or eukaryotic 28S rRNA.
- The method of claim 3, wherein the rRNA comprises the sequence of SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, SEQ ID NO:43, SEQ ID NO:44, SEQ ID NO:45, SEQ ID NO:45, SEQ ID NO:45, SEQ ID NO:45, SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:56, SEQ ID NO:57, SEQ ID NO:56, SEQ ID NO:56, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:78, SEQ ID NO:86, SEQ ID

- The method of claim 1, wherein the sample comprises eukaryotic nucleic acid.
- The method of claim 1, wherein the sample comprises prokaryotic nucleic acid.
- The method of claim 6, wherein the prokaryotic nucleic acid is from a gram positive bacterium.
 - The method of claim 6, wherein the prokaryotic nucleic acid is from a gram negative bacterium.
- The method of claim 1, wherein the bridging region, targeting region, or capture region comprises at least 10 nucleic acid residues.

10

25

- The method of claim 9, wherein the bridging region, targeting region, or capture region
 comprises at least 15 nucleic acid residues.
 - 11. The method of claim 10, wherein the bridging region, targeting region, or capture region comprises at least 20 nucleic acid residues.
- 20 12. The method of claim 1, wherein the bridging region or the capture region is polypurine or polypyrimidine.
 - 13. The method of claim 12, wherein the bridging region is polypurine and the capture region is polypyrimidine.
 - 14. The method of claim 1, further comprising incubating the sample with a second bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the second bridging oligonucleotide and the targeted nucleic acid.

92

15. The method of claim 14, wherein the targeting region of the first bridging oligonucleotide is complementary to the sequence of a targeted nucleic acid and the targeting region of the second bridging oligonucleotide is complementary to a different sequence of a targeted nucleic acid.

5

- 16. The method of claim 15, wherein the targeting region of the first bridging oligonucleotide and the targeting region of the second bridging oligonucleotide are complementary to the same targeted nucleic acid.
- 10 17. The method of claim 15, wherein the targeting region of the first bridging oligonucleotide and the targeting region of the second bridging oligonucleotide are complementary to different targeted nucleic acids.
- 18. The method of claim 17, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of the largest rRNA molecule and the targeting region of the second bridging oligonucleotide is complementary to a sequence of the second largest rRNA molecule in the sample.
- 19. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 100 and 5000 residues of the 5' or 3' end of the targeted nucleic acid.
 - 20. The method of claim 19, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 150 and 4000 residues of the 5' or 3' end of the targeted nucleic acid.
 - 21. The method of claim 20, wherein the targeting region of the first or second bridging

- 22. The method of claim 21, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 250 and 2000 residues of the 5' or 3' end of the targeted nucleic acid.
- 5 23. The method of claim 22, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 300 and 1500 residues of the 5' or 3' end of the targeted nucleic acid.
- 24. The method of claim 23, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 350 and 1000 residues of the 5' or 3' end of the targeted nucleic acid.
 - 25. The method of claim 24, wherein targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 400 and 900 residues of the 5' or 3' end of the targeted nucleic acid.
 - 26. The method of claim 25, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 450 and 800 residues of the 5' or 3' end of the targeted nucleic acid.

30

- 27. The method of claim 26, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence located between 500 and 700 residues of the 5' or 3' end of the targeted nucleic acid.
- 25 28. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence at the 3' or 5' end of the targeted nucleic acid.
 - 29. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence not within 100 residues from the 3'or 5' end of the targeted nucleic acid.

94

- 30. The method of claim 14, wherein targeting region of the first or second bridging oligonucleotide hybridizes to a sequence not within 200 residues from the 3'or 5' end of the targeted nucleic acid.
- 5 31. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide hybridizes to a sequence not within 400 residues from the 3'or 5' ends of the targeted nucleic acid.
- 32. The method of claim 14, wherein the targeting region of the first or second bridging oligonucleotide comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:81, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86.
 - 33. The method of claim 1, wherein the bridging oligonucleotide comprises a second targeting region comprising at least 5 nucleic acid residues complementary to a different sequence than the sequence to which the first targeting region is complementary.
 - 34. The method of claim 33, wherein the first targeting region is complementary to a different targeting nucleic acid than the second targeting region is.
- 25 35. The method of claim 1, wherein the first bridging oligonucleotide comprises two bridging regions.
 - 36. The method of claim 1, wherein the bridging oligonucleotide or the capture oligonucleotide is RNA, DNA, LNA, iso-bases, or a peptide nucleic acid.
- 30

20

37. The method of claim 1, further comprising washing the capture oligonucleotide after incubation with the sample and the bridging oligonucleotide.

- 38. The method of claim 1, wherein a) and b) are performed at the same temperature.
- 39. The method of claim 1, wherein a) and b) are performed at a different temperature.

- 40. The method of claim 38, wherein a) and b) are performed at the same time.
- The method of claim 1, wherein the nonreacting structure comprises a bead comprising plastic, glass, teflon, silica, a magnet, cellulose, latex, polystyrene, nylon, cellulose,
- 10 nitrocellulose, polymethacrylate, polyvinylchloride, or styrene-divinylbenzene
 - 42. The method of claim 41, wherein isolating the targeted nucleic acid away from the sample comprises exposing the sample with the capture oligonucleotide to a magnetic field.
- 15 43. The method of claim 1, wherein the nonreacting structure is cellulose.
 - 44. The method of claim 1, wherein the nonreacting structure is biotin.
- The method of claim 44, wherein isolating the targeted nucleic acid comprises incubating
 the sample with streptavidin or avidin.
 - 46. The method of claim 1, wherein the sample, capture oligonucleotide, and bridging oligonucleotide are incubated in a buffer comprising TMAC or TEAC.
- 25 47. The method of claim 1, further comprising:
 - discarding the portion of the sample that hybridizes to the capture oligonucleotide.
 - 48. The method of claim 2, further comprising:
 - d) discarding the targeted rRNA nucleic acid; and
 - e) producing cDNA using mRNA in the remainder of the sample.

- 49. The method of claim 2, further comprising:
 - amplifying nucleic acids in the remainder of the sample, wherein the remainder of the sample is enriched for mRNA.
- 5 50. The method of claim 49., further comprising:
 - e) using the amplified nucleic acids to probe a nucleic acid array.
 - 51. The method of claim 48, further comprising:

10

- n attaching the cDNA to a solid support, wherein a nucleic acid array is created.
- 52. The method of claim 51, wherein the solid support is plastic, glass, or nylon.
- 53. The method of claim 52, wherein the solid support is a plate.
- 15 54. The method of claim 53, wherein the plate is a multiple-well plate.
 - 55. The method of claim 48, further comprising:
 - f) contacting a nucleic acid array with the cDNA.
- 20 56. The method of claim 1, further comprising incubating the sample with a second bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the second bridging oligonucleotide and a second targeted nucleic acid.

- 57. The method of claim 56, further comprising incubating the sample with a third bridging oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the third bridging
- 30 oligonucleotide and a third targeted nucleic acid.

- The method of claim 57, further comprising incubating the sample with a fourth bridging 58. oligonucleotide comprising (1) at least one bridging region comprising at least 5 nucleic acid residues and (2) at least one targeting region comprising at least 5 nucleic acid residues, under conditions allowing hybridization between the targeting region of the fourth bridging oligonucleotide and a fourth targeted nucleic acid.
- 59. The method of claim 56, wherein prokaryotic and eukaryotic rRNAs are targeted nucleic acids
- 10 60. A method for depleting rRNA from a sample comprising:
 - a) incubating the sample with at least a first (1) bridging oligonucleotide comprising a bridging region comprising a poly-purine region of at least 5 residues and a targeting region comprising at least 5 contiguous nucleic acid residues complementary to a sequence of an rRNA molecule and a (2) capture oligonucleotide comprising a magnetic bead and a capture region comprising a poly-pyrimidine region of at least 5 residues, under conditions to allow
 - hybridization between the bridging oligonucleotide and the capture oligonucleotide and the bridging oligonucleotide and the rRNA: incubating the sample with a magnetic bead; and
 - b)
- 20 c) isolating the magnetic bead.

15

- 61. A kit, in a suitable container means, comprising:
 - a) a capture oligonucleotide comprising a capture region and a magnetic bead; and
 - at least a first bridging oligonucleotide comprising (1) at least one bridging region b) complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.
- 62. The kit of claim 61, wherein the first bridging oligonucleotide comprises a second 30 targeting region.

- 63. The kit of claim 62, wherein the first and second targeting regions have the same nucleic acid sequence.
- 64. The kit of claim 62, wherein the first and second targeting regions have different nucleic
 5 acid sequences.
 - 65. The kit of claim 64, wherein the first targeting region is complementary to a sequence of an eukaryotic rRNA and the second targeting region is complementary to a sequence of a prokaryotic rRNA.
 - 66. The kit of claim 64, wherein the first targeting region is complementary to a sequence of an eukaryotic rRNA and the second targeting region is complementary to a sequence of a different eukaryotic rRNA than the first targeting region.

- 15 67. The kit of claim 64, wherein the first targeting region is complementary to a sequence of a prokaryotic rRNA and the second targeting region is complementary to a sequence of a different prokaryotic rRNA than the first targeting region.
- 68. The kit of claim 61, further comprising a second bridging oligonucleotide comprising (1) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.
- 69. The kit of claim 68, wherein the targeting region of the second bridging oligonucleotide
 is complementary to a sequence of the same rRNA as the first targeting region.
 - 70. The kit of claim 68, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of the largest rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of the second largest rRNA in the sample.

15

20

- 71. The kit of claim 68, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of an eukaryotic rRNA and the targeting region of the bridging oligonucleotide is complementary to a sequence of a prokaryotic rRNA.
- 5 72. The kit of claim 70, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of an eukaryotic 28S rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of a eukaryotic 17S or 18S rRNA.
 - 73. The kit of claim 70, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of a prokaryotic 16S rRNA.
 - 74. The kit of claim 70, wherein the targeting region of the first bridging oligonucleotide is complementary to a sequence of an eukaryotic 28S rRNA and the targeting region of the second bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA.
 - 75. The kit of claim 68, further comprising a third bridging oligonucleotide comprising (1) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.
 - 76. The kit of claim 75, wherein the targeting region of the third bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA.
- 25 77. The kit of claim 75, wherein the targeting region of the third bridging oligonucleotide is complementary to a sequence of a eukaryotic 18S rRNA.
 - 78. The kit of claim 75, further comprising a fourth bridging oligonucleotide comprising (1) at least one bridging region complementary to all or part of the capture region of the capture oligonucleotide and a (2) at least one targeting region comprising 10 contiguous nucleic acids complementary to a sequence of an rRNA.

- 79. The kit of claim 78, wherein (i) the targeting region of the first bridging oligonucleotide is complementary to a sequence of a prokaryotic 16S rRNA, (ii) the targeting region of the second bridging oligonucleotide is complementary to a sequence of a prokaryotic 23S rRNA, (iii) the targeting region of the third bridging oligonucleotide is complementary to a sequence of a eukaryotic 18S rRNA, and (iv) the targeting region of the fourth bridging oligonucleotide is complementary to a sequence of a eukaryotic 28S rRNA.
- 80. The kit of claim 61, wherein the first targeting region of the bridging oligonucleotide comprises SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:11, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:85, or SEQ ID NO:86.
- 81. The kit of claim 61, further comprising a buffer comprising TMAC or TEAC.
- 82. The kit of claim 61, further comprising a magnetic stand.

10

- 83. The kit of claim 61, further comprising:
 - c) a solid support for preparing a nucleic acid array.
- 84. A bridging oligonucleotide comprising a (1) bridging region comprising a
 polypyrimidine or polypurine stretch and a (2) targeting region comprising at least 10 contiguous
 - nucleic acid residues complementary to a sequence of an rRNA.
 - 85. The oligonucleotide of claim 84, wherein the rRNA is eukaryotic.
- 30 86. The oligonucleotide of claim 85, wherein the rRNA is the 28S rRNA.
 - 87. The oligonucleotide of claim 84, wherein the rRNA is prokaryotic.

101

- 88. The oligonucleotide of claim 87, wherein the rRNA is the 23S rRNA.
- 89. A method for depleting or isolating a targeted rRNA from a sample comprising:
- 5 a) obtaining the kit of claim 61;
 - incubating the sample with the bridging oligonucleotide under conditions allowing hybridization between the targeting region and the targeted rRNA;
 - incubating the bridging oligonucleotide with the capture oligonucleotide under conditions allowing hybridization between the bridging region and the capture region; and
 - d) isolating the targeted rRNA from the remainder of the sample by incubating the sample with a magnetic field.
 - 90. The method of claim 89, further comprising:
 - e) obtaining the remainder of the sample enriched for mRNA;
 - f) preparing cDNA from the mRNA.
 - 91. The method of claim 90, further comprising:
 - g) constructing a nucleic acid array with the cDNA.

20

10

- 92. The method of claim 89, wherein the mRNA or prepared cDNA is amplified.
- 93. The method of claim 92, wherein the cDNA is used to probe a nucleic acid array.

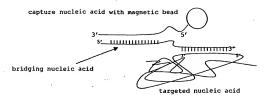


FIG. 1

-Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

The same of the sa	10	20	· 9-	- g.	- 0
Banthracials, sm		c	6		1
Stacealle 165.520	- CUMOCE - COTTON - C		b		
Llactis 165.830	-77247776		· Terral circ-	1.0.00.0	65
	000-1000000-0.00.00.00.00.00.00.00.00.00.00.0	98	5		
Saureus 168.530	717 - 20100	300	6		
Smitans168.520		5	5		
Spneumon165.820	55		6		83
Spyogenes168.520			5		53
Mavium168.820					0
Mt.b168.830	25	***************************************	5		25
165.830			6		64
	TRANSMITTANTONICAMITTANTONICAMITTANTONICAMITTANCONICAMITTANICAMITT	TCATGGCTCAGATTGAAG	-500000000000	CCTANCACAT	CCANOT 62
	99	. No.			33
SEO	09				
nchiseptica 168.82	Ø				62
spertuesis168.820	Bpatspertussis168, 2227.7			1.1.	
Spertuesis 168.820	35				35
	£9		0.1.		61
	63				
udomellei 168.880	10. 10000000000000000000000000000000000				37
Mgonorrhoese 168.520	Mgonorzhodes 168.570 Therement of the control of th				77
Mening 165.830	£				
8	69				63
Vcholerae 168.8EQ	9900002120		8	CCTTC	99
and and the last owner	C7	•••••••••••••••••••••••••••••••••••••••			9

FIG. 2A-1

Allgnment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	20	ě-	· 8-	- 70-	- 61	. 62	- 81	140	- 87	9	170	l
Baubrille168.520	GG. Ab G-C Argr C CAGO C 2AA. CT C. G C. G C. G 186		C.CATG	T, C.			0	S. AS		0.0	8.0	
Zfaccalle 168.520	A.T A. Marin C. A. Marin C.		CT. A MC		:			CAZAAC		C.G	C.00.	164
Liactis 168.822	7. G. C. G. KO. Till	Company	The same					0		5	۲.	53
Lannocyt168.8EQ		9	OT.CCAMO			3 8						
aureus 168. SEC		CAGA	CATC	FC.		8	Ų	ATA			8.0	ž :
Spreamon168 gro	Q.G., CL. OD. MAC. G. CTOT. MACCONSTITUTE CTT. C. A. C. CO. M. T. C. M. A. C. G.	C.CTO	ACACCOTOT	T.CTT C.		C.89.A	.T. C.	AT.A.C.		,	, ,	1 5
Dyogenes 168 . 820		T-LOOK				c.83.A		G.A.C.			Α	162
Navium168.880		9	10 C C C C C C C C C C C C C C C C C C C			c.83.		1 C A.C.			ΥΥ	2
(tb168,530	31	9		1		8				300	7.007	=
coll ol57 168.820	COLUMNIA LOCALIA LOCAL	100				8	2		۵	35	7.007	270
Consumentes 168.880	G CAC			The second second	Compage	ASTANTOTOR		SCHOOLOGIC	GOOGATAGE	ACTOGRAMO	COTACCTALTAC	173
Asctino168.820	997		3		:							91
Hinfluenzae 169,820						5			,		T. C	173
onchisentice 168.	545.0					ŧ			g			175
Barabertunista ern n c n c n c c c c c c c c c c c c c c						٠	8			8	60	ŝ
Beartman 165 870 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0							8		9	8	0.0	142
Beenacia 168.580				,			8	C. A.C.	9	8		P
Bmallei 165.5EQ						9.00	đ (7.Cld.A.7	ğğ	00	COOCUT	E
Spandomalle 168.820	0			O			5	T.C.C. A. T.		9		3
genorrhoese 168.830			8	0		5	8	0.0			Sec. AT.	
Destructions 160 cm			8			CA.A.C	8			0	7,10	16
Choleres 168.880						c.		G.A.T.	99	J.C.	8	
**************************************	880.0.0.0.0.0.				*			C.0.A	9		A. G	Ł
						:	•	**********				h

FIG. 2A-2

Alignment Report of Gram +&-165 align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1 3		. 6	- 00	- å	-	220	å	. 05	380	9,7	. 270	280	İ
Boubt111a168.5EQ		OTTOTAL	20.04	OTTOMO:	1	1	ŀ	ŀ	2 3000	-		1	1	1
Benthracisto. 520	9	A.TT	1.3C.OC.7	COTTC.M.	3	8	G A. TITG. AC. G. T. GITC. A. TITGAA. GGT. GGTGGT. TIN	1		•		•		9 6
Efaccalls 169.5EQ	:	Y COL	1.0.0.1	COUNTY.	TOWN	8	ACTT: T. C. CC. T. COADAA. OTGANA. CCTT. COATGAT. TOAT. CA. COCCT. C. A. C.	đ	2	•		•		2
Llactis 168.5EQ	:	1	C.AMC.	TTTOW T	TCM.	TOCAL.	ANN TIT. A. JC. TITEMA TITEMA ATOCIA. TO CALA COLOR TO TAKE TO THE TAKE THE	A.T.	8	١		1	•	
Lannocy t168.830	4.:	LTMT.	7.0.0.7	. OCOC. CTT	TOWN	TOOT.	A - 10. EAM. TOT 0. CC. T. CCAC. CTITICANA. ATGOTT. GOCTATC. TIP. A . G. COCOT. C.	o	cooper c	£		ŧ	•	
Saureus168.520	9	Ę.	1.30.00.1	OTTOMY.	TOMA.A		O TA. TITO. AC. CC. T. GITCHAA. GGD. 10-CTOTCA. TIATA. GAT. GGGT. C.	đ	5	ŧ	1		4	
Smitans168.820	:	.n. m.	TATTOC.1	. ATAMTT T	TCMA.A	TOCHO	TA-ZAATTATTOC, T. AZAATT. TTGAAA, AGGOAG, G-CATCA, GA, TGGA, GB, TGGA, T. T. A.	đ		•			•	
Spnemon168.820	•	9.00	7.7700.1	. ACATTE CE	TANK.	100.1	GAGT-G-7.TTGC, T. ACATTT. CTTAAAATGCATG.—CATCA. F. CA GBTGCG T. T.	ð	7.200	£				
Spyogenes 168 . SEQ	:	4.400		TAL TATE	TUM.	COCH.T	GAGA. ACTA GG.T. TTA. TDAUTTANAN. GGCAN. TG.—CTCCA. T GA GA	ð	7 2 2000	ŧ		1		
Maytumi 68.880	9		8	remeno	TOURS.	F	.00046.1		200000	•	٠	ŧ	•	
Mtb168.820 .		SOC.A.C	100.10	7077. 706	TOTAL ST	5		4		•	•		•	3
Boolf ol57 168.52Q	200	ACCRECE	MODEL	A-MOCCOUR	0000	į.	COCATANOTICAL MACENTAGE COTTORES CTCT TCCT MACENTAGE MACENTA		1000000					28
Kpneumoniae 168.820				•			-		TWO INTO	A4.146146	4	AKE TO THE T	ACCCACCAT	38
Aactino168.520	9	8		5	ķ		277	•					:	277
Hinfluenzae 168.530		1	2	0	1000		-	•						.0. 284
Bbronchisestica 168-820	9	77	9	,	į		0	•				5		.1. 286
Boarapertuania168.820			8	,	١,			3			:	*		0 281
Bantusais 165 FED Annual Co. 1.1.1.1.00001.5				,				ğ			:	5		6 253
Benneta 168.5EO				,		•	CAS #1.00 CT . C	8	3.T.C.		:	٠. t		6 279
Beallet 168.520		1		,		: !	CAL 177.0 - 6. 100-7.	2	5. TOOCT.		:	4		30 282
Roseudomailed 168 SPO		1		,			CON MIT WITH THE PROPERTY OF T	P	7. TOOCT		:	¥5		255
Wondy 169 0wo								5.5			::::	¥5		35
Mening 168.000				,	:	ĺ		8				ACA		.NG 287
Patricines 168 APO				,	:		Control of the Contro	8		•		٨٦		.AG 287
Webolance 160 min			•		•			4			:	٧٠		0 283
Ventavoor 1 to 1 to 1		: 1		Ş		ĺ	284	4			Α	4		284
						i	A K.	:						

FIG. 2A-3

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

INDEPENDENT STATES C.C.A. 10.70.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.0.	Cold 0, 170	COMP. 01.750 02		٥			-			-		
	000000000	00000					1	0 0	1			3
	00000000	6000		Ü								
8	000000	000	•	,				;				ş
R	00000	0.0	,	•					•	8		ä
e g	00000	o	,	,				8		88	.A.C	3
~ 8	0000									£	A.C	Š
a 8	000		:			:	¥	.0.0			A.C	į
8	0 0					:	¥	8			A.C	3
}					:	:		8	A. 0	68	.A.C.	8
					:	:	A	98		88	A.C	80
						:					0.0	163
Zenii niti ika can anomone						:		:	•		909 · · · · · · · · · · · · · · · · · ·	30
	Market Inc.	THE PERSON	A CONTRACTOR	NO.	AGICTOCIA	2000000	OCHOTOGOGN	TATTOCAC	ANTGOOCOGUA	OCTUNIOCA	THE	90
	•	•				•		:		:	260	197
OED.			,			:		9	3		70°	504
Onchiespeice 168 820	,			•		:			N 0.0		909 · · · · · · · · · · · · · · · · · ·	90
Commonthage and Common			;									5
Destructed 168 220	7					:			J		6	5
						:				c.	660 · · · · · · · · · · · · · · · · · ·	ŝ
			,									8
028.8				;	:				:::::::::::::::::::::::::::::::::::::::		3.12	138
	•			;							359 · · · · · · · · · · · · · · · · · · ·	54
	•					:					104	6
928	•	•	,								407	ê
	•										to 1	3
								:			709	ş

FIG. 2A-4

Alignment Report of Gram +&- 18S align.MEG. using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

SECRETARY SET 16 TO THE	:	625	420	.430	ş.	- 55	_	- 9	670	480	69		200	510	å	
	1168.880		¥	·C	G. TOTTA.	4	3	C-COTTOD-	.00000.1	A.C.	0	3	8	-	1	3
0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	168.820	2 9			O. TOTTA.	4	3	C-1.01.0	, AOC. G.	A.C		6.00	8	4		8
A	. 085.891	2	ŧ	ţ	27.07.0		į	TO A CO		8 9		9			:	. 52
4444	66.88Q		¥		G. TOTTA.	4	3	7.0				3 5		.	:	2
A A	029.83	9 9			.0.T.TA.		SEST	TOTA.C	OTOCA.	Ä	ē	200	8			ŝ
A	68.520	2			A TOTAL		e 1	20.0	5	A0	.00.	ra.coc.	1.0.6.	Α		5
	168.820	92	£	ŧ	1000		3	9.00	9			5	.0.0	Υ	:	3
	.820	88		ŧ					1		. A. D	9	.0.0.		:	ğ
	g	7. 68		į						8 8	8				:	÷
	17 165.530	TATGLACIUC	CONTROOR.	TOTAMOTAC	TTCACCC	and an	8	OTAN CITTAIN		9	.0			٥.		4
	tee 168.820						8	0.0.4	0		•			ATMATICAL PORTING		2
	8.820				0.TAT.	:	F		G. A. GC.	2	2					:
	DEC. 202 . 202		:		0.TXT.		F	ATGT	G.ACAT.	2	-W-	5				: :
					2	λ.λ	ě	G000.c.	.T.C.930	5				4		
	. 160 000	8 8			4.9	γ.γ.:	ÿ	CACCO.C		5				4		1
	168.870				9	A.A.	Š	CLOSG.C		5				Α		š
	68.520					, Y	2	x.ooctc	X6.C.G	100	9			¥		3
	llet 168 gan						1	9		8				4		\$
	025.550 app						Ę	1.010		ğ		۲			:	\$
. 2	68.520				5		1			3				¥	:	5
	BR 168.830	c					Š	C. 671.C	1.700.0	3		F.A				8
3	168.830									6	7	3		-		3
***************************************	olitica 168.8	D.G.		,	•		F 1			3	•					8
				:		:	5			8						:

FIG. 2A-5

Alignment Report of Gram +&• 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	ŝ	240	220		960	970	280	- 86		- 00	- 8	620	- 63	ı	- 9
Beubcilisi68.520			T. T	٤			1		,		,		- 0	F	7
Stancalle 165 cm			77	į.		:						9		P	7
Liactis 168.570							9	:					0	2	7
Lennocyt168.880			# ## ## ## ## ## ## ## ## ## ## ## ## #				9			7.0	8		ATT.T.TO	P	7
Saureus168.5EQ			T											2	7
Smitane165.820		•	T. T.C. O. OTT T		٠	•		•				٠	000	ģ	7
Spneumon168.520	:		7. 170.00 OTC 7. 7. 17. 170.00 OTC 7. 1. 170.00 OTC 7. 170	μ	•		9		·		8 8		ATA. TOTO	2	í
Spyogenes16S.SEQ	:		TCC. G		•		2				9 8		8.1.1	2	1
May Lumi 65. SZQ			7	2		1		f	ŧ		•			2	:
Meb168.880	:		162 - 101. MOCH 101. The second of the secon	2				,	į				101	e e	:
Ecoli ol57 16S.SEQ	OCCOCOCOCAN	TACCGRACO	OCCOCOTALIACIONACOTICALACOTILATOCIALITACIONI ACTOCALACIONA CONTRA ACTOCALACIONA ACTOCA	VICOS.	TACTOR	COLUMN	T. Contract				-	,	.TOT. MOCG.	8	:
Koneumoniae 165.520	:							,	į,	,		TO THE PERSON NAMED IN	COLOGONCIA	Ž	á
Asctino168.882			36 3c		4	·	•	<u>ا</u>	,	;	:	•		ģ	ė
Hinfluensae 168.5EQ		2	099 = ''.'		•	•					:	•		j.	:
Bronchiseptica 168.8	02	ŧ	DO				١	•				•		Ü	ö
Branchine fales. EDO							2		3	:	:			4	•
20- 10- 10- 10- 10- 10- 10- 10- 10- 10- 1							2	:	3	:	:			4.4	- 3
Bornacia 168.570		,					P		3	::::	-			4.4	:
Emallet 168.820			369 40'-'01'-'01'-'01'-'01'-'01'-'01'-'01'-'	,			2	:	۲.		:				9
Speeudomalle: 168.530			119 YD:	•			P i	:			:			g	٩
Nganozphosse 163 820		•		•			P 1			;	:				٩
Mening 168.880		•	7				3 8		9					5	ø
Pasruginose 168.SEQ		1	A. A				3		1 5	9 1	:	:		6.7	ø
Vcholerae 165.520							;							đ	ż
Vantercoolitica 168.5ED	02		0000 - 10							:			ATG		÷

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

					I	I						
	8	099	. 6.	. 08	069	- 00	- 62	. 62	. 6	. 09.	750	- 92
Baubtilis168.520		18	A.A. 0.	22			0	٥	1	7.10		16.14
Banthracis16.820	G. C. A. 1777 T. 7777 A. G. C. 761	CCA. A.	MA. G				4	4	4	77. 7. 70	4	.00
Ztaccalis 168.520		8CA.A.	A.A0			•	4	4			2	0 0 753
Llactis 168.820		OCH. 0	A.A. G.			•	4	U	4	DT. C. T.	1	0. 0.769
Leonocyt165.520		SCA.A	A.AG				0	٠,٠	, V	7.7.70	2	0. 0. 780
Seureus 168.820	A	OCA.A					4	C A.	, Y	TT TO	5	.G.T. 770
Smutans165.520		OCA.AO	A.A6			•	Α.	C A.	,	7.7	2	.00 769
Spneumon168.5EQ		OCMO 0	A.A. G			•	Α		,,	r.T	2	.00 756
Spyogenes168.520	- AG. A. G. A. A. G. T.	OCA.AO	A.AG				*		4	F.T 7.70	2	00
Mavium168.88Q	.ddh. h	ACT.C0	A.AC.0				Α		6	T.T G. AG		G A 711
Mtb165.820	.ddkhkccgkkcdcdc	ACT.C 9	A. AC. G.				Α		6	F.T 0 . AG		A 752
Zcoli o157 168.88Q	стосиместамистоткамососотимитескететиссетамиссетимистимисторует 163	CTCTTACAGO	DODOTMONT.	TOCOGOTOTA	GOCOTONA	TOCOTACAC	VICTOGADON	ATACCACTOCA	CONCESSOR	COCTOCIO	CACTOLOGI	TOWN 762
Konsumonias 168.520	. d. A									Α		754
Aactino168.820		ACTT. N. G. J.	N							T		. 7.60
Hinfluenses 168.520		ACT 0.3						***	4	TANDT		762
Bhronchiseptica 168.830, Ac. GG. Ac. GG. GG. Ac. GG. GG. Ac. GG. GG. Ac. GG. GG. GG. Ac. GG. GG. GG. GG. GG. GG. GG. GG. GG. G	Q.AC.00A	0.0TC	AG	8		*	8	CA	¥	T	,	T.C 757
Bparapertussis168.889A60A6AGGAGGAGGAGGAGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGGAGGGAGGGAGGGAGGGGAGG		0.000	AGA.	8			8	C	4	7. 00.7	Ü	T.C 729
Bpertuesis 168,520	- AC.03 - A G.07C A G	G.07C	Α G.	8	Α		8	,	Ψ	7	Ü	7.0 755
Boupacia 158.820	6 . h. h. d. C	A.Q.C			Α			Α	4	0	ı	T.C 758
Beallei 166.550	6 . A A. G. E	A.O.C.		,				γ	4	0.0	ı	7.0 711
Bpseudomallei 168.520		A.O.C.						4	4	0	1	T.C 810
Monorrhoese 168.8EQ		G.0TC	A0.	Ü	4			4	4	5	Ü	T. T. 763
Mening 168.830		0.000	7				0	4	1	7.000	Ü	T T. C 763
Pasruginosa 165.530	. ACTO A ACTO			1.1				.CA	4	4	5	757
Vcholerae 168.680	. A	101					A			2		A. 760
Vanterocolities 169.820	O		:				N			*		763

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, Novembe 27, 2011

	110	. 09.	. 8.	. g.	810	. 82	830	- 98	_	850	. 09.	870	- 8	
Baubeilis168.SEQ	λατά, .λλ. π. τ. τ. λ. α. αστ c - ασο. ε. τρώ, ε. τ. τ λ. Ν. εμετ. ε. ε	9				NOTE		1.0.00	888	0.002.3	2.0.2	γ. γ.	15	188
Banthracis16.5E2							X. 7.7.	A.A.057.	900	T. TM. G.	T.A7.	4	5	80
Zfaecalis 168.8ZQ	7		:			MOTO	M.T.T.	9.A.OOT	8	0.00.1	T.C A	4	5	20
Liactis 168.520	7		:::::::::::::::::::::::::::::::::::::::			AOTO	A. AT. TM	0.000.Th.	4.4.	C.CTO.AT	Ü	2	5	188
Lannocyti68.820	2.1 COCC					YOUR		A.G.00T.	8	0.000.0	2.0	4	5.00	66
Saureus 168, 830						NOTO:	. Y. T. T.	A.G.OOT.	8	0.047	0.5	4	50	88
Smtans168.820	TT	40				AOTO	A. T.T.	A.OCTC	8	C. TAG. G.		2	5	888
Spreumon168. SEQ	T					DIOM	A. 7.7	A.ACT	8	T.TM.0.0.		4	50	375
Spyogenes 168.830	TA.CC. T. C-CG. OC. TMG. GA						. A. T.T.	A.00T.	8	C. TMG.G.				3
Mavium165.820			:		N	0001.	AT.	Q. TT	1	88		4		=
Mcbles. SEQ						6	AT.TO		6	9.88.6		4	8	5
Ecoli o157 165.5EQ .	GENANGCOTOGOGACIAACCHGAITEGALCCTGGTAGTCCACCCCTIAAACCATGACACTTGGAGGTTGTGCCCTTGAGGGGTGCTGAGGTAACCGAAACTAAATGAAC	ancensor	GATTEGRATACE	CTOSTAGE	COCCOSTA	UCCUTOTODA	CTTOOLOGT	TOTOCOCTT	98-46	procer	COCHOCTANC	DOCTEMA	TOOLOG	8
Kpneumoniae 168.820	RI-CO					•						-	-	22
Asctino168.820						9	T0.A.	6.0	0	785	γ.	4.5		9
Minfluenzae 165.530	T G. 077						40	10.0		9	-	4		
Bbronchiseptica 168 874						Å	. A.C.	9	į	0.7.0.70			E	20
Dparapertussis16s 8EQ A	Α					Α	A.CT.T.	9.00	8	C. T. G.MG			7.	8
Spertussis 168.53Q	A*					***************************************		0.0-0.	8	C. T. G.NG				22
Bospania 168.820	A				C	Ψ	.A.TT.T.	0.0-CM.C	i.	C. 23G.33				375
Scallet 165.530						Ψ	A.T.	0.0-CAT.C	5	C. 23G. A.				848
Sprendomallei 168.520	Ai					Ψ	. A. TT. T.	0.0-QAT.C	M-11.	C. 202. D		8		22
Monorthoese 165.820						Ψ	T.A.C. T.	6.6CM	P	C.T.G.M				881
Meening 168.500	C			:		4	T. A. CT. T.	G.OCM.C.	1	C. T. G.MG				881
Pasruginosa 168.520		:					, A.R.	0.Q.T.		C. TM. 00	8.0.9	****	:	973
Vcholerae 168.820				:		•		•	1	•			4	878
Yesterocolitics 168,682,	2	•		:			:		į			:	:	880

9/54

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	88	8	910	950	930	96	920	. 09.	970	980	086	- 60	
Baubtilis168.520	7 1000 A 1000	ļ	C. 0.	0								ľ	00
Banthracis16.880			.C.0	0							4	•	8
Efaccalis 168.520	066 E	,									4	•	8
Llactis 168.8EQ	λ										4	4	00
Lmonocyt163.530		Α											019
Saureus168.5EQ		A						1			12	•	8
Smitana168.830	300 C T T T T T T T T T T T T T T T T T T							:	,		4	Ü	80
Spaeumon168.52Q	366 D							*******	į		Α.		2
Spyogenes 165. SPQ	60 5	,						***************************************	,		Α.	2	s
Mavium168.820					N	0	************				5		::
Mebi 65.530							Y				6		22
Ecold e157 168.5EQ	GCCTGGGGAGTACGGCCAAGTTAAAACTCAAAATGAAGTGGGGGGGCGGCAGAGAGTAGAAGTTTAATTGGAAGAAGGAAG	accoun	STEMMETER	WIGHTIGHT	COCCOCCOC	CAGOOOTO	GACATOTOCI	TEATTOOK	DCMCCC	ACMOCTING	CTOSTCTTON	1000	1000
Kpneumoniae 168.520	Constitution				***************************************							Ţ	83
Asctino168.520	966 D						:				, VC	0	8
Minfluenzae 165.520	000 a				N					:	g		86
Baronchiseptica 168.680	8		,		····	:	g				8	8	8
Sparspertussiai68.5EQ											99	ğ	996
Spertussis 168.820			,		¥		2					8	22
Bospacia 16ff.5EQ					····		2					8	8
Bmalle1 165.8EQ			A							Α		8	898
Spacudomallet 168, 520			,				2			· · · · · · · · · · · · · · · · · · ·	200	8	1047
Ngonorrhoese 168.820						:	2		:	:		8	1001
Menting 168.820						:	, p		:			8	8
Pasruginosa 165.530	582 T.D					:			F			1.0···	3
Venoterse 168.820			•	:		:				:	Ç	:	8
Yencerocalica 168.322	2	:					***************************************			:	Q	:	8

-IG. 2A-9

Alignment Report of Gram 48-16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1010 1020 1030	1040	1050	1060	1070	1080	1080	1100	1110
Baubt111s165.5EQ	-70 .AA.C.T A. OAC CT 700 . G - 1 2 T	B. G. A. P.	,		-	1			
Banthracis16.830	-TG -MACG-T	2 40-12					:		8
Bfaccalis 163.520	7-70. COC. 7 A. ACTY, C. C. TOCC. MM. T 6 1117	444.0							0 113
Liactis 168.520	- GTGCTRT-C-7A.GAA.T-GC-TGTGG. AC. G7.	P. 0 - 3	e					•	
Lancocyt165.520	T-70. CMC. TO CA OCTT. C C. TOO. 1-0. AAA. T	2 0 0	c	,					
Saureval65.820	2-70 -AM. 1 A00CT. C00	D G MA. T.	t	,					C11 0
Smitana168.820	G. TOCTAT. CTT A. GAA. T. AC. TOC. TAC. T.	3.7.C.TC	c	ŧ					
Spnevmen168.520	1776. COC. 1 A. G. 17. C.—C. 100	2. AC. QAT.	c	,		;			
Spyrogenes 168, 820	0.100.000.1A. 0.171.A6.1700.100.170-1.	2.20.70.0	c	,					
Mavium165.5EQ	0C-000101041.0001.C.100	The Comment				;		:	
Mcb165,820	0C-GONCT GMT. GOC. T. C. TT.		,					:	907 0 2 708
Zcoli ol57 168.5EQ .	CACANG-TROCOCAMAGAIT CONTRACTOR C	OCCUPATION OF THE PARTY OF THE							
Koneumoniae 165.520	1117		ACCIDENCE OF	100101001	CACTION	TTOTOTOTOTO	TOGGETANG	receses and a	SCICACCOCTA 111
Agetinol68.830	A. Ch. Ac.	•		,			:		330
tinfluentee 168.580		Ē				:			
Shronchiseptica 168.	Bbronchiseptica 168.8200 TC-con.								111
Sparapartusaie168.52	to TG. TC-CCA. TTCCCA								6 111
Spertuseis 165,520	Spertuses 168,520 TG				:				907 0
Bospacia 168.820	.0. TC-C.5. G. TOTAL . C. C								0 111
Bmallei 168,820	G. GC-COLE GREEN B. C. CO. CO. C.								111
Spseudomellel 168.520							:		2 708
Mgonorrhoese 168.5EQ									0 116
Moening 168.830									111 0
Paeruginoss 168.52Q	0 1118	7 7					:		
Vcholerae 165.8EQ	0TCNd.0C.CTGGA,	0.0							
Yenterocolitica 168.	Vanterocolities 165.5200 . 7 M						:		TT
			:	:	:::::::::::::::::::::::::::::::::::::::	:::::::::::::::::::::::::::::::::::::::			:

Alignment Report of Gram +&-16S align.MEG, using Clustal method with Weighted residue weight table. Tuesdav, November 27, 2001 4-14 DM

	1120 1130		1140 1150	1160	1170	1180	1190	1200	1210	1220	1230	
	£A	A- T. A.	NR. A	9	00			1	, C			1232
•	JA3	A TM.	KTAT.AEMA.TTCTT.	9								1235
8	70. A	A	TG. AT. A TTATCT. GCC	9		:					:	1225
•	TO. A	.ATA.	TG. A T. A TAA. TT C T C				:				:	1242
Lamonocyt168.88Q A	T. A	A	ATT. A			:		Α				1250
	GA	A T.	NO. A					Α		777	:	1246
	70. A.	.ATA	TO. A	9	λ				.C		:	1243
•	76. A.	A . T. A.	TG-A	5	AC				.CT		:	1230
028	70. A.	A TA.	TG-ATATAA-TTCT-G-AG-AC		Α			A				1138
ogs	10	0. ME	TCA	9.5	rccrc				.CT.1		4	1186
	10	.ACM.T.	TCA	9.9	c. crc				C T		4	1227
3	CONTRACTOR	DOD-01000	INCITIVITIESCHAGE ATCORCCOARMATCHANABICHCHOCHARRANICTORNOAMOTOOORITAKOATCATCATCATCATCATCATCATCATCATCATCATCATCA	COCOCIO	TOUTANGETOO	DOCUMENTO	DOLITOROGIC	MOTCATCA	COCCUTACO	CCADOCCTACA	CACOTOCT	1236
. 820		T.	f.: 122								,	1228
Aactino168.520	6	-1001	.66	9						8		1232
Hinfluenses 165.850		CTT								5		1234
Bhronchiseptica 168.5EQ.A.ATAAACT.TGCC	1.A. A T.	7	W C T I						T.		5	. 1226
Bparapertussis165.8EqAATAAACT.TG.T.CCCT.TCT.G.T.CC	A.A	-	W C T T	6.	T.C C				J.4	201	5	1198
8	. A. A T.		.A. A. T						y.g		5	1224
Bospacia 168.5EQ	AT.	J							y.2	7	đ	. 1227
Beallei 165.5EQ	AT.	1		0					J.T.	ZTT.	ð	1200
Bpseudomallei 168.5EQ .	AT.	3).E	707	3	. 1279
3.820	. A. A.	T.	.A. A		CQ.C						5	. 1236
	. A. A.	C.AT.A.	.A. A T.AT.A.TTCT. TGCG.CG.C1236	9	CQ.C						3	1236
8	AA	900		5								1231
Vcholerse 16S. SEC	6									5		1235
Venterocolitica 168.882		. M M.	5			:						. 1237

Alignment Report of Greim +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

Bsubt111s163.SEQ				2			1300	or.	9.	2	1340	1350	
		0.0	MONTH OF CONTRACT ON T. CA. T. C. A. C. TOTOT. TC T. C. C	1.6	.CA.T.C.A	CTCTCI	7	Ü		0.0		ľ	1351
Banthracis16.520	2004			9000	P.T.	A0001	76.7	5		•	f		ž
Sfaccalls 165.520	OWO		QANG	6	. W.T T	3	2	0		c			
Clactis 165.880	ATOD		**************************************	TOTAL.	P.T.	XOCX1	ę.	e.	Ü	4			
Lannocyt168.520	ATM.		AZAO	9100	.T.T.C.	ACEN	2	•	·	•		į	
Saureus168.SEQ	YC.	3.6	ACA	8	A.T.C.		į	•		•		: '	
Smtans168.820			1000 (TT 0.00.1	8	TA. T.	80	¥	c				•	
Spneumon165.820	100		T00, CTCA. 0C3 TA. T CCA TC. T.	8	7.4	8	4	F	,	•			
Spyogenes 168.820	1100		TTGG CTCA.G.GJ.TCGTA.TTCCAATCT	8	TA.T.	3	ų	e E		•			
Havium168.880	8	0.0	CO3 1905 TA OTT A T COTT CON TO A	6	•	E	į		!		•	; ;	
Mt.b168.820	88	3.0	CO3	ţ	A.T.C.	8	į	c				5 6	
Ecoli o157 168.880	CATTOCCOC	BOWNSON	CANTROO CHICAMANA MARCALCO CONTRACTA CANTROO CONTRACTA MARCAL CONTRACTA CO	CHARACTUS	COCOCCTC	ATAMOTOC	TOTAL PROPERTY.	CATHERING	1000			5	
Uneumoniae 168.820	AT		Set Division in the set of the se			A.T.					-	e se	9
Actino168.8EQ		00		8	T. A. T.	0	ŧ					•	
(influenzae 169.820	•	0.0	T	COTO.		•	ŧ					•	
bronchiseptics 168.870 10000 6.40 6.00 6.40	820	0.0.0	C. C. A. C.	8	5	2		;					3
Parapartusés186.820	2001	2.0.0.1	70. C.A.C.	8	5	0							5
pertussis 168.520	2001	300.1	10000 0.00 0.00 0.00 0.00 0.00 0 0 0 0 0	8	5	9	×					•	:
Bospacia 168.520		00	TODAN	8	77.0	. 300		ú		Ì	,	•	
Emalled 168.8EQ	1000	1.0.0.1	TOURN. G. G. T.C. C. A. C G	.00	5	9		ú					
pseudomallet 165.530		1.0.0.1	TOGGA G. 9.TC C. A. C	0.0	5.7.0	.O ACCC		Ü			f		
Monorrhouse 168.820		00			5	.c	;	0		0		. 1	
mening 168.830		00		0000	5	.c				0.0		ð	2
Peeruginose 168.820			T000 6. TT. C. ACC F. GG TA. T. C ACCGA C. C 6		D.1.0								23
Venoting 108.680		9.0			¥.	,						5	1354
enterocolitica 198 SEGAdTAAA		•	4	:	¥	5						•	135

Alignment Report of Gram +& 16S align.MEG, using Clustal method with Weighted residue weight table.

	1360	1370	1380	1390	1400	1410	1420	1430	1440	1450	1,460	1470	
Baubtilis16S.SEO			-	1	1	1	C	ł				1	:
Banthracis16.550	0	0				v	1	8		CHAME .	Access.	200	1473
Efaccalis 163.5EQ	Υ						A. T. A.	8	CC0	THE .	AGOC		1449
Llactis 168.520	Ÿ							2.08		acM	2	7,00	1478
Laonocyt168.820		0					ATA.	8		OTATT.	A.AGCCA., COC	0.700	1487
Saureus169. 830				4		0	ATA.	ä		TTTMC	LADOTA, COT	9,700	1483
Smutana168.520	Υ						ATA.	8		DAKTT	1,000.A000	7.700	1481
Spineumon168.8EQ	Ÿ .						ATA.	8	C. +C. · · G. · · · · · · · · · · · · · · · ·	GMG	3. ACCCA CCC	9	1466
Spyogenes168.8EQ		6			:		G	ä					1338
MAY LUMIOS . SEQ	20.0	2				5	.M C M.	8	G. AA. OCCCA. G. C. C. C. AA. OCCCA. G. C. C. C. C. C. TII. G. AG. AA. A. G. AO.	F	3.20305	0.700	1426
menta						5	WC	ğ			3. AG. A GT	0.700	1464
779:987 / 518 11000	WICKEN S	ACCEPTANCE OF THE PARTY OF THE	TACATTCCCC	ACCTTOTAC	CACCOCCCO	0000	ocumocorre o	3	WITCHAM THO THE WATCH CONSIDER THE TRANSPORTED FOR A STRUCTURANGE AND THE TRANSPORTED FOR THE PART OF	ACCTTCOOC	100000CTE	COCTET	1472
Applementae 188.850		• 1					:	:	100			:	1464
Medianotos. 30						:		8	A	eee		8	1468
The contract of the contract o							:	8		£		8	1470
DEPERTMENT OF THE PROPERTY OF								8 8	•	8	8.	8	1462
Spertussis 168,820	Ü	6		ı	2		•		200 V. 1000 V.		8 8	8 8	1636
Bospacia 165.530							1	8		S	1	8	1461
Beallel 165.820							4	8	2	8	0	8	1436
Bpseudomalle 165,580								8.5	6663666	8	.0-A-0.	8	1515
Monorrhoese 168.520		A. 70				:	8	3.6	.gch.rg			8	1472
menting 108.820		P. 1			:	:	18 0	8	. doc. = . A. 24. 24. 24. 24. 24. 24. 24. 24. 24. 24	ACM		8	1472
Water uptroom to S. Bry		. 1				:		8	A	3	J A G	8	1467
Venoterae 165. SEC		P				:		:	A		X		1466
**************************************			:	:		2	:	:		N		:	1473

IG. 2A-13

Alignment Report of Gram +&- 16S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

Raubet 1 fa 1 69. 920	1480 1490 1500 1510 1520	
Benthracisi6.320	.a. c.	1427
Lisctis 168.5EQ	Ab. CCC.	1449
Saureus 168. SEQ	.dCAA?	
Smurane168.520	.ggog.	1555
Spyogenes163.530	YD	1515
Mavium168.52Q Meb168.620	.dcoc	
Scoli ol57 165.5EQ	OTICATION TO CONTRACT AND CONTRACT AND CONTRACT.	1536
Accelerate 108.820	A	1534
Hinfluencee 163.820	A	1485
Shronchisaptica 168.	Bhronchiseptica 166.529.0	1487
Sportugeis 169.820	Particle 166 3E0	
Brepacia 168.520	AG	1464
Parile: 168.920	Уд	
Monoschart 160 mm		
Manual 168.830	TOT	
Paeruginosa 168.520		1544
Voholerae 168.520		1537
Yenterocolitics 165,5EO Na. N.	N. 184	1467
		1485

Decoration 'Decoration \$1': Mide (as '.') residues that match Ecoli o157 168.5EQ exactly.

ı

- Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table.

Tuesday, November 27, 2001 4:14 PM	7, 2001 4:14	PM											
٠		- 97-	- 8-	oğ.	- ş -	- 95	- 8-	۶.	- &-	- g.	30.0	l H	
Baubtilis 235.820	TT.GA. (0.09) TT.CA. (0.100) TT 10T. (04. (02. (0.100) A.H.C. T. T. (04. (0 A.C. AC) TT. T. A.	.T	8		ACT. O	8	G. ACG. A	9	8	A.C.M	70.7		111
Benthracis 238.850		11.9	8	T.	9		G. ACT. A.	K	8	A. T.AG	C TO. T	7	:
Efacaells 238.550			8.		9	8	O.XCT.A.	£	.1.00 .C.	A.T.M	C. ATO. TA	A 4	=
Llactis 235.SEQ		7.7.	3.8		ACT. ACA.	8	XCF.A.		20.00.cm	D. T. A	CA. 75. T. C	0.0	=
Improsoytogenes 215,522		5.	8.5		9		G. ACT. A.		.T00C.	DV.T.O	80.TG.T.		11
Saureus 235.SEQ		11.1	8		-ACTA	8	TACT.A.	¥	.T. 30C.	A.T.AG	C. TO.T. A	7	1
Smutans 218.820		T.A. T.	8	4	9	8	¥89	34C.T	.T. 80. C.		CC. TO. T.	A 1	110
Spneumoniae 238.520		7.1	8		. G		XC	J 4	.TQC.	A.T.AG	CCATG. TA		=
Spyogenes 235.520			8.8		4	8.	Y-154	P A.T 3	.38	A. T.M	OCCT0.7		
Mavium 238.880					4.00	8	8.8		8	TC.ACCOAC	CA. 70. T.	9	67
Mtuberculosis 238.530			8.9		A G.C.	8	8.8		8	TC. ACCOUNT	2.00.00		12
Eco11 238.520	***-d***Accelctalacetalacetacatecalacetacalacetalacec-atalacetectalatetecealalacetecalalacetalatealacetralacesear	CONCTAG	COTTACACACATO	2ATGCCCTGCC	AOTOMO	300-ATGMCC	ACOTOCTAATO	POCENTANDO	TOOUTHOOT	CATATOUCK	XXXXXXXXX	DOCAT 1	12
Kpneumoniae 218.520					:							-	3
Minfluenzae 235.650	FATA T A A T A T. G. A. G. T. G. A. G. T. G. A. G. G A. G. G T. G. A. G. T. G. A. G. G A. G. G A. G. G A. G. G A. G. G. G A. G.				¥	A			.O.A.O. T	8		3	13
Bhrochiseptica 218.880	- Y C		F.C.T.T.		3				D000	.0C.MC.X	CA.70.7.	5	11
Sparspartussis 235.5EQA-C	3-W	***************************************	7.C.T.T.		3		30.00		G80C	.0C.MC.M	C 12.1	5	112
Spertuesie 238.520	***.**********************************	:	T.C.T.T.		 5			A	8	.8C.MC.K	CA. 70. 7	3	7
Bospacia 235.520		ç	1.0.101		9			A	A804	.0C.MC.X		1	77
Beallet 235.520		9	1.0.101		3			A	88	.oc. Model	70.10	1	4
Bpseudomaile: 23s.Spg ===.==GACF.G.TOTT.GAGG.G.GACTAGG.G.CG.G.CAATAGG.G.G.		ý	10.101		9				38	OC. MOOM	X . 76.7	4	112
Ngonorrhoses 238.520			1.C. TCC.		6.0.7		£.00	4	8.00	8. A K	C.ATG.TT.	-	77
NewInigititate 138.5Ep===CTAcadata. T.C.TCh			7.C.TC.		6.6.7		TA.00.	y	9.08	.0C.ATA	CAATG.T	-	
Paeruginesa 235.520		7			:	AA		A	8	9.0X.90.	19.3	7	3
Vcholerae 235.620			:		:				9.7.	30. A. O.	5.5	-	77
Yenterocolitics 215.5E@,	20		:	¥	:							-	2

1G. 2B-1

Alignment Report of Gram +&-23S align.MEG, using Clustal method with Weighted residue weight table. Tueaday, November 27, 2001 4:14 PM

	120	051	7		150	160	- 57		180	- 61	- 95	210	220		
Baubtilis 235.8EQ	CONCTOS ANT GAGTOS CA. T ATA, G. GA AZ G AZ	OCACTO	3	CACTOC.	5	•	*	TA.G.GA.	ν			6	0	1	•
_		OK. A20	9.1	9.18		4		TAA-OOA.	7. MG C			5		O A 23	_
Efacaelis 238.820		A.A.CT	7	MOG.T.T.	Ė	٥	ÿ	B.T.O.	20.00	Α.Α		7	9	GAA 23	_
Llactis 235.530	C	6	7	70JOT.	70.0	G	Ü	C.O.W.	75	Α.Α		32		GT A 22	
Leonocytogenss 219.520	0	C.A.C1	2	000.1.0	0	GA.	8	. OC01	30			2		CA A 23	•
Saureus 238.520	CA. M. AT. TOT. GA. TO GA. TO GA. TO GA	5	9	Ė		G	5	F.C.9	,			9		. 64 4 23	_
Smutans 238.5EQ)	λ··········· 6	NCA	ž	0	5	7.4.		J. 0.G.	.MG0C	A.T		C0.TOC	9	06 22	_
2		XCX	3	. TOT	.000.7	4	1	10.6.6	200.00	A.T	:	6.100		GA 22	
g	9	a.c	3	2	5	4	ì	G.0.G.	200.00	A.T				OA 22	
Havium 238.830			9	6			:	тосоосис.	8			6	0	AT 23	
Ntuberculosis 238-889		66.	3	ě	C.00C.T	¥		TGCCGCAG	8			6	8	AT 23	
Ecoli 238.8EQ	TTOCOM/TOCOAN/CCCAGTOTO-ATTC-GTCACTATCATTAACTCATACATA-TAATGAGCCAACCCCCCAAACTGAAACTGAAAGTAGTCAATAAAAAAAA	ACCCAPTOTO-	Ė	grencia	FATCATTA	CTGMTC	NEWOOT	TAATCAC	COCMOCO	COCCANCTO	MCATC	PAGEAGGGG	POCHANGO	MICMC 33	
Kpneumoniae 238.820	G	5	}	2		A			:					33	
Hinfluences 238.830		AGN	3	AATCT	ý			5	4	Α				33	
Shrochiesptics 138.8EQN:		38	I	8	8.8	4	:	C-MOTO				C O. T		33	
Bparapertussis 218.5EQA		8	į	9.0	8.8	Y		C-MORE:	:			C0.T.		333	
Spertussis 235.SEQ	A	8	į	9.6	8.8	۲		C-MOTO				C 0. T		323	
Bospacie 238.880	.9	80.0	į	0.001	5	٠		C-ATGC.,	8			8			4
Emalle, 238.5EQ .	6		į	10001	CC.MG.	4		00	8			W			5
Bpseudomallat 215.5EQ 9			į	T000T	C C.MG.	*****			8			y		225	
Ngunoribosse 235.550 0			ì	1010	C.A.6	4k	•	3	,	Α		0		33	80
22			Ì	1010	C.A.6	۲	¥	5				0		33	-
3	C.TGGTA.G. AA-CCTGGT.GT	8	3	8	5	:	:	8	1		:	F			
Vcholerse 238.82Q			3	ţ,	9	•		200	7.7		:				9
Vanterocolities 219.528gC		đ	ì	P	TOCAT	γγ		ATOCA.							

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

Baubtille 238.5EQ		240	250	36			. 270		280	ı	
	J.C.	2	8	C-10.0	-TCA	GAACCA	AG. TTOOCT. T-	TOSTISTACCAC	T.C. 10. COA.C.AC. CA.TCA		
Senturacia 238.580	4.C.			7	CATA	AACCA	AG. THOOPT. T.	OCCUPATION OF THE PERSON	T.C. T. TO		
Efacaelis 23S.SEQ	Ë	2			-AGA	Ch	CA. TRECTION	GGGTTGTAGGAC-	TTC TO TO		
Lisctis 238.500	MC	F.OTA		CA	-GAA	3AACCA	A TREET T	GOGTTGTAGGAC	NAC TOTAL CONTRACTOR C		
Lannocytogenes 235.5EQATC	2ATC			-MCM	-AGA	GAACCA	A TTOTT T	COGREGATION	-C. Th. AC-		, .
Saureus 235.620	£	r		9	-AQAGQQ		CA. TTGCTTGT.	COCHICTACCAC	TTC		
Smutans 238.83Q	A.C.	3.7		3	-003	CNCCA	W.T. 777.C.CT	GGGTTGTAGGAC-	h.C. 16. T. 17. C. 16. ChCa. ChCa. 17. C. 17. C		
Spneumoniae 235.520	¥.C.			3	-GA	CAAACC	A. TITL CICING	COOPTIONACCIAC	A.C G. 17.	,	
Spyogenes 238.520				5	-00y000	CI	TT COC TC	OCCUPATION OF THE PROPERTY.	. C		• •
Mavius 235.8EQ		6		3	CHACTAMOCOCATO	CATGOLOGIACO, GOT.	G. T. TOT. T. TOC	COGREGATION	T.T OT C-C MCMOOTAMOODATOMOODAG. OF THE GOTTOMORANIAN TO CONTINUE SEC	AT CHARGE 16	
Mtuberculosis 235.520				8	CHOCTAMOCOCACO	CATOGOTIANOC. GOT.	0. TTOT. T. TGC	- COCTTOTOCOMO-	T. T	700000	
Scoli 236.SEQ	FINDS	DOCCOLOTERO	COCCUPACO	COCO-Y	***************************************		CONGCOCAGAGGCT	1	COMONT TOCOCOLOGIA COSO		
Konsumentas 238.520					***************************************						
Hinfluensee 235.520		5			*************************	A	A AGT. AGTO	J	T.G. M.	30	
Bbrochiseptica 238, 820		3	4	1,014	************	***************************************					
Dasabetuesis 238.620		70		227			•				
Spertussis 238.520	:	44			***************************************				700		
Bospacia 238.520		***		1		***************************************	î î				
Brallet 238.580		***		-	***************************************		2		A. T.		
Bpseudomallet 236.520	:	**	4	-AE.	***************************************		4		100		
Mgcnorrhoes 235,320		d.A.	4	1.0	*************	***************************************	2.2				
Monthstatie 335.500		A.0.									
Paeruginosa 238.520							Ē		T. 11.	787	, .
		58		b			1		787 LL	200	٠.
Vanterocolitica 115.520	8				***************************************					87	

Algoment Report of Gram +&-23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM.

	0,	25
	60 37	Control Authority Control Cont
	340 50	1. 6. 100 mm. 6. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.
	320 330	10.00 A.
-	310	24. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6. 6.
	30	Character 12 Carrent 1
	Baubrilla 218 sec	

FIG. 2B-4

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

		١,	ŀ	-	1	ŀ	-	ŀ	ł	ŀ	+		-
•		200	9-	610	420	ĝ-	64.	\$ 20	99	670	480	690	
Baubtilis 215.580	A		.0	A	2		6.6.			-	0	A	844
	4	U 0	0 0	,	2 8			:		•	0	A. M. M. T. C. O. C.	. 545
Llactie 238.820		4	8	-	8								2
genes 238.820	A		8	4	¥					•	Ü	253 Annooy't cogames 236.5EQ.A. AA. T.C A. CC. A. TC. CT.O.	23
Smitene 336.822	2					•	7.0	:					3
920		,			5 t			:			•	253	623
920	A. O	Ł	0	4	8					• •		AC.AATCGA	8
		8	c	8	.03	į.	8	ů	£	•	•	. 0. C A	3 3
338.820	.0.0	8	8	88	6		8	0.0	ŧ	6	f	.0.C	2 5
Ecola 438.8EQ	anchorage	DECEMBER	CTGMATATO	aaaaaccaa	XTCCM00CE	MATACTCC	талатала	ATAGTON	CONTRACT	CHOCHMODE	MANAGA	аменатольноститетам титалого систем, поста	8
	1		4 0				•				:		669
8	4		d			•	1				:	bkrochisaptica 218.880	202
tusete 218.880	4		G								:	Sparepartuses 218.350	192
Spertuseis 238.520	7	;					¥						2 :
	2	;	0				ħ					25. D	2 5
Brallet 238.620	2			:		•							6
Monorthone 210 cm	•				:		¥.	:			:		191
Litais 238.580	0						¥ .					Nemingittedia 23.550.0 . A.	00
Pasruginose 238.830		E	Ü			1						. M. M. M. M. CTT.	66
Vcholerae 235.520	4											000	2 6
Mittee 215.52	3											enterocolitics 218.5EQAC.	6

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	200	510	220	230	540		250	260	870	580	230	99	
Bsubtilis 23S.SEQ	0		0	Ď		07	1	2	0	WC.0	8	Į	
Santhracis 238,520	F		0.0.	DXIC	C.G	97.,		9	0	MC.G.	8	5	648
Sfacaella 238.820		:	0.0	ð	A			.0	0			0.77	9
Jactie 238.830			0	AT	C.O	fc		9	0		88	. M.	533
monocytogenes 238.820	£		0.6.	ğ	· · · · · · · · · · · · · · · · · · ·	·		TA0.			.0034.7	7.7	647
Saureus 238.5EQ			0	5	L			100	0	MC.G.	8	. V	
Amutens 238.880		:		A	c.o			9		. MC.0.	8	50	632
Spneumoniae 238.820		:	0.0	5	c.o			G G.		AAC.G	88	AT O	633
pyogenee 218.820	•			5	99			6.			.0CGA.A	-, AT. 0.	Š
tavium 238.8EQ				0.0.5	rc.00		8	g		.A.C.T.	4000.00	0.0.0	698
ftuberculosis 235:5EQ			a.c.	D. D.T.	F	dT	100		Α	. A. C. T.	40000	V	20
Scoli 235.5EQ	CHANGE	CTONNOC	STATISTICS	CUICCIOTO	XMACCICITIE-	GAMANAMOCTANACOTATION CONTRACTOR	PATOGOGIA	CTOCOTACC	ACTATOTET	TOGOTCHOCK	DCTTATATTC	T-CTAGGAR	5 604
(pneumoniae 238.5EQ						, AC . G							9
Kinfluensae 235.880						.T							į
brochiseptics 238.882777	20		A.O.A			***************************************					c		2
Bparapertuseis 135,5207	707		A. G. A.	, A		***************************************		c			,		
Spertuesis 238, 520	1		A.0.A.	Α	•	2		e					:
Scepacia 235.550			.C.0.A.	4	9	310		۰			3	,	
mallei 238.8EQ			C.0.2.	Α	1		8	0			8		:
Speeudomaile: 218.SEQ			.C.0.A.	Α	1)	200	0			8	4	9
**************************************			TCA.0.A		8.0.					1	t		
eminigititais 236, 550 C. TOA. C. A A C. C. C A C. C. A C			TCA. 0.A.		8.0.	7				4	Ü	9	9
Paeruginosa 235.820			A.O				4.1		:			٨ ٥	25
Jeholerae 235.850				¥	ACC-			:				A 0	8
Variation 118 680 C					•								

:1G. 2B-6

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table.

	g.	820	630	079	059		9	099	670	989	- 69	
Baubtilis 238.580	9.	0.10	0 0	0.0	٥	0. 3.1. C.	5			1	1	į
Banthracis 238.550	0	0.0	0	6	ľ	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0					; ,	1
Efacaelis 238.8EQ	5	Q. O	Ü	ď	7	(2) (2) (3) (4) (4) (4) (4) (4) (4) (4) (4) (4) (4						2
Llactis 235.SEQ		9.0	F	į.	Ş	07.1.0.00						
sonocytogenes 235.52	0.0	A A C	c L	ć	į	Leonocytogenee 115.520 0.0 A.C. 77 0.0 A.C. 77 0.0 0 0.0					:	į
Saureus 238.520	d. A.	7.7.4	T. C. D	2		G.A.T.A.B.T.		3 2			: '	1
Dautens 238.552		0.30		9		0	1			: '	: :	
Spneumoniae 235.520	6	9. 0	f	ř.	g	0						٠. ن
Spyogenes 238.880		9, 0		Ę	٠	71. 0. AT				, ,	;	ξ.
Kavium 238.820			0.0	2			,				;	;
Mtuberculosis 235.520		10.0T.	0.0	AT. O	30		2445		•		: :	: :
Zcoli 235.53Q	OTTAN-COO	WINDS-OOK	COMMERCANICO	GAGTETTAACCO	COLUMN	GT2A-COMADIG-GRACEMATENT CONTINUES OF THE CONTINUES OF TH	No.	1000				
Agnesmentae 238.820	********		U	•								3
tinfluenzae 238.530				t	7	Cas State 336 305 Cas						:
brochiseptica 238.81	200	A.	0. TCA.A.N.O.	8.73		Bbrockiseptice 235.5200	Ų	f		1		•
perapertussis 235.51	20c		G. TCA.A.N.G.	8	4	Destabettussis 235.8200	,	•			;	
Spertussis 235.520		4	O. TCA.A.N.O.	8	Ĭ	C	,	f			;	
Bospacia 238.830			00	8		000 J		8		:		
Bmallei 238.8EQ			07.0	8		C		8				:
pseudomallei 238.820			0.7.0	8		Dpseudomilat 218.820 C		8		:		
gonorrhoese 239.5EQ	· · · · · · · · · · · · · · · · · · ·			4	4	Ngonorzhoese 235.5EQ C						•
eminigitatedis 235.51	DOC.			4	4	Neminigistads 235. SEGC					٠	
Pasruginosa 235.820			G. T. C G.	đ	·	G		,			:	•
Vcholerae 235.850			T.C. 10	•	Ü		t	4				•
'enterocolitica 235.1	SEC			8	4	Yentercolitics 238.5ED						:
									:			

Alignment Report of Gram +&-23S align.MEG, using Clustal method with Weighted residue weight table.
Tuesday, November 27, 2001 4:14 PM

	-	2-	65.	92.	740	750	92	5.	780	790	8	. 01-	
Baubtilis 238.880	0		9.		0.8	8.0	8	G.A.		9		•	862
Banthracis 236.820			. O. A.				8	G.A.C.				:	863
Efacaelis 235.820			8	· · · · · · · · · · · · · · · · · · ·		8.6	88	G.A.C.		. A.G T.			826
Llactis 238.820			8		conc.c	6.91		A.A.C.		A.G			827
Lannocytogenes 215.520				J		30.0	88	G.A.C.					863
Sauraus 235.520		T.Ch			3.6		, X	A. G. A.C.		0		•	862
Smutans 235.550			8	***************************************	CONC.C			G.A.C.	A	A.GT.			94
Spneumonies 235.830			8	***************************************		100.0		G. A. C.		A.GT			\$
Spyogenes 215.8EQ	:	GT	8	***************************************	MODOC.C	0.021		G.A.C.	A	A.GT	F		83
Mavium 235.880	0			3		0.0.0.	99	0.A.	:	•	8.4	:	8
Mtuberculosis 238.520				,	T.G	.0.0.0.	8,			F	X.7.	:	3
Ecolt 238.520	CHARTTENA	ChartehagrigathachcthaltoshaacoshctbatottaaaAattbacoshtacttataactososhtaaaaocaatchacosaakakoctootteteee 817	CTARCTICA	STATESTANCE	DETERMENT	MANANTENG	COGNICATI	TOSCTODOS	TOTOTOTO	MICHIGO	DOGMANDOCT	postation	8
Koneumoniae 235.520		\$180 \$100 \$100 \$100 \$100 \$100 \$100 \$100					***************************************					:	2
Hinfluenese 238.530		600					•		:	:		:	83
Bbrochksptics 235.820		90		***********			8	A.A			T A		8
Sparapartusis 215.552		9		***************************************			8	A.A		AT.	T		8
Spertussis 238.830	:			***************************************	6		99	A.A			T A		8
Bospacia 235.530	Υ	. A	5				8	A.A			T A		18
Bmalle1 238.820	A		6	*****			8	A.A			T		8
Egseudomailei 238.5Eg	y	8	6	7		0	8				T		=
Ngonorzhoese 135.552		8				8	98	G.A	-				8
Neminigititdis 215.520				********	8	88	88	A.A		F			8
Paeruginosa 238.8EQ				1	ooc	8		A.C					8
Vcholerae 235.8EQ			3	:									8
Yanterocolitica 218.500	9												8

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	830	830	840	850	99	870	-088	990	906	910	920	930
Baubtilie 235.520	TAGC	, ATS	¥	**************************************		1.4	ACTA. C.		G.ATTC.Q.	0	2	3
Banthrecis 235.5EQ	2000				A	A-4.	ACTA	CTCATGA	.O.ATTC.G.			T. C A
Efacaelie 238.680	200	AT-SO	4	"SACC CETA. G-MATTEMEN ATENT AT. ACTA C. C. T. GO G-MITE OF C. T. SO ATTC. T A 973	.A.		ACTAC.	8	O.ATTC.Q.	5	O. AT	TC 7.
Llactis 238.8EQ	2002	OCTA.	QMA	TABLE G MANDEMANDENTED A T G	.A		STG.	8.75	D.77.T.	5	W.T.O.	DYC
Landscytogenes 115.880144C	TAGE		4	OT. MO. OTCAT.	A	5	ACTAC.	C1.1.00	.O.ATTC.Q.	,	0A.	A
Saureus 238.SEQ		OCTA		TMC			ACCAC.	8.1.8	G.ATTC.G.	AC.	W. 0	£
Smutans 235.520	1300	OCTA	0		.A	7	TTCA	CG	1.10,0	77	4	4 05
Spneumoniae 238.820	2000	ac.	9	240C 000Th. 0 ACATT. 040. TOTT. A7 07Ch	A	*	orga.	8	9.7.1.	£	W. 0.	4
Spyogenes 238.620	100		0		A	5	OTO.	9	9.57.5	, c	W. 0.	A 45
Maváum 235.820		8	0.7.0	**********************************	AT.	0-AT.	CONT. C.	C-, A, TAGO,	.TGOTC.0		0	0.70.AA-A
Mtuberculosis 235.5EQ		8	9.7.6		A	C-AT	CCATC.	CA. TAGG	.TOOTC.O.		0	4-M-01.0
Ecoli 215.882	COLOCTAT	TRADOLOGO	0.0000	GANGCENTTHAGENGOCCTOST-Q-ANTTCATCTOCOCCOCATALAC-ACTOTTTCOCCAACACACACTTACCAACGAACAACCAAATACCAAAAAAATATTTT- 932	2000CTACAGOC-1	CTOTATOO	CAAGGGGGTC	-ATCCCCACTTA	CONDOCAL	CAMCTEC	CANTACCOC	AGAATOTT
Rpneumoniae 235.530										4	4	
Hinfluentes 235, 530	•	A.	1		-		7			4	***************************************	
Bbrochiseptica 215.520			N	T.T-T.CTO.A.				8	A. A.	v	•	50.0
Dpsrepertuseis 218-820	A			T.TT.CTG.A.	•			8	AATG			C. 0. AC-
Spertuesis 238.5EQ	A				•		+	8	AATG	0	1	CA.0.AC
Bospacie 238.880	A					5	TTO	T. 70. ACA.	000 . ATA	0	4	4-00
Erallei 235.6EQ	A	-					130	T. TO. AGA.	ATA	v	•	4-0.0-0
Bpseudomalled 218:580	y	-		TCTCA.C.T		5	110	T. TO. ACA	ATK000.		•	4-0.0-V
Mgonorzhoss 238.5EQ		-		C. GA. CTGAT	A	AT.	1	T. TO.A.	DIV	5	77	6.0.0
Neminigitiedis 235.880				C. GA. CTGAT				T. 10.A	DIW	3	M	0.00
Peeruginosa 238.820							+		Α	9	ฮ	0.0.0
Vcholerse 238.830	:		3	. 6-4-CGAAT.CTA.T		***			£	0	4	TA. 0. AC. A
Vanteuronality (no. 218.82)	5									•	•	

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	940	95	950		970	980	08.	1000	1010	1020	1030	1040	1050	
Baubtille 238.820	75.77	7	TC-272	1	8				200		1	-	1	į
Banthracis 235.620	TC. TTA.	7.0.7	TO THE	4	٥	G							:	
Cfacesiis 238.520	TATE	1.0.1	DAC . 7. 0. 7		8	c								6
Llactis 235.880	TOTTC.	.0.4	7077 . C. T. O. T. T		8	e	1						-	1089
Improcytogenes 238.82073	EQTA.T.	0	9 Y . Y .	1	8		4		A				:	
Saureus 238.820	Υ.1	7.0.10	A. 77	ė	4 50		4						:	801
Mutens 238.520	.c.c.	0.1	046		8	•	1						:	6
Spneumoniae 238.520	TOOF C.	7.0.7	7007. C. T. G. T. T. C.		8	G	1						•	1 6
Spyogenes 215.SEQ	TAMEC	7.0.7	TAKTCT0TGLAA-	,	8. A.	0	4		1					
tavium 23S.SEQ	6.6	10.0	0.07. C. 170.0	χ	8.A.	0						0.00	• :	3
(tuberculosis 235.5EQ		9.0	-6.07. C. 176.00	×	8. A	c	•		to to		,		:	
Zeeli 238.5EQ	ATCACCOCOLC	CATAGO	ATCHCOCOLACATION CONTINUES OF THE ACTION OF	-STOCOLE	CTANCACTO	CARACTAGO	TACATA TACA	AGE TABLETON	The same and					
Uneumoniae 218.520			DSG TOTAL CONTROL CONT				-	-	-		TOTAL STATE OF	Commerce Co.	1	8
dinfluencae 238.520	46		.C	•	c				•		•	• 1		9
Brochlessties 218, 820, 0 TT.	E0.02	0	c	ŧ						:			:	8
Darabertueste 218.8	100			•					. T. ATC		*		:	1038
Destruets 238, 320 G TT	ŀ			5					TATE		4	¥	:	ŝ
Scepacia 238.820			0.24	5 6					. G. T.ATC.				-	ŝ
Brallei 238.520		c	O AT	ŧ					C. A. ATA.			4	-	Š
Dasaudomallat 215.520	g	c	0 AT -					•		,	•	4	-	1045
donorrhones 219 cm				'							¥	4	7	1045
minimitated as a second	a Javan		20 T	•					TOTAL				:	50
Paranicipus 216 erg r m		•							101		¥		:	801
Vaholense 110 cm		:				:	:		P			F	:	90
5002			100g	•		:	:	:				•	:	8
The state of the s														

	1060	1070	080	1080	1100	1110	1120	1130	1140	150	1160		
		λλ		8		8	Ę	0	0.4			I	1211
	L.T	A.TCAY CUC		5	•	8	ţ	٠,	AT.	4			17
Efacaelis 218.820		A. T. A. T. A. A. T.		6	•	ð	9	4	•	į			300
Lisctis 238.880	A.T	λ. Τ		8	•	ð	9	٧.	4	Ę	Ĺ		1201
238.820		•		5		8	9	٠.٠٠	4	Ę	,		
		A.TCACACACACAA		5	4		5	A	4	Ę	ŀ	Ę	1
	Y	A.T		6	4		g				7	ğ	1200
Sprogener 235.880		ATT A C C C A C C A C A C A ATT T ATA. A. A C A ATT T ATA. A. A C A ATT T ATA. A		6 8	•		9 9		· · · · · · · · · · · · · · · · · · ·			4	1202
	0	A 0 A GATT T. A.	0	8		4	, Table	4 2	< 0			4 6	2 2
238.820	Α	A		6		4	ATTG.	C. 73	0.0	ý	ם נ	3	2 2 2
	accidantario	осийскі прітості на месь сем тем местем претем претем претем претем претем прем претем	CATTOM	AMOCOTA	ENOCEOCUE	producto	accrac	COGNICATOTA	ACCIDENTAL CONTRACTOR	-OCHOCOGN	CTGCGG		1167
		1108	:	:		:					•	-	165
Hantiuensee 235.820				:	•		:			Α		-	5
EXPOSITE 23 SEC. T					:	į			0.0	A-A		-	55
pperpercess 23.8 mg Tr				:		-			.0.0	γ-γ	:	17.75	134
Special 238.880										A-A		11.5	727
Beallet 238.880	F	T. C. C. T. NAN-								W.	•	5	1160
38.880	0									- WW			1162
Mgonorrhosse 238.8EQ		and the control of th											7077
Q							f						2011
8		21. 1			1				0	1			128
Vcholerse 238.820					-					E.		1	132
1991							:				:	-	199

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday November 27 2004 4:44 584

	1280	The Author The
	1270	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	1260	0.03 0.03 0.03 0.03 0.03 0.03 0.03 0.03
	1250	60 00 00 00 00 00 00 00 00 00 00 00 00 0
-	1240	A TOO
	1230	(3), , , , , , , , , , , , , , , , , , ,
	1220	20.00 (20
l	ii.	1,000.00 1,0
Į	1200	
f	1190	7.7
4 14 7	1180	00-M21 11-A0-A0-A 11-A0-A0-A 12-A0-A0-A 13-A0-A0-A 13-A0-A0-A0-A 13-A0-A0-A0-A0-A 13-A0-A0-A0-A0-A 13-A0-A0-A0
12/ ZUD	1170	11.7. AND
Luesday, November 27, 2001 4:14 PM		Sequences 218 EQ

Alignment Report of Gram +&- 238 align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM.

	ŀ	I		-	ŀ	١	ŀ	ŀ	۱			١				
	1290	ä-	1300	1310	1320		1330	1340	1350	1360	1370		1380	1390	1400	
Baubtilis 238.550	ÿ	4	10.7		700	80.0	t	.	8		1		إ		ľ	
Banthracis 238.5EQ	44	A	7.0.7		100	8	t	ŧ	8	200 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	,	,	:			
Efacasiis 238.880	7.1.1			ŀ	8	8	8	ŧ	8	.T. T.	·					1448
Llactis 235.550	T.TEMC.		•		A.000	8.0	8	ŧ	á	.T.TDXC.ATTA.003.16.03.C.C.T. T. 001.			\$		•	
Lannocytogenss 235.520		A A	TAT. T.		80	80.0	t	e	8			,		9	•	
Saureus 235, SEQ	7	A AT			7	8	5	f	Ş		,		:	; ;		
Smutene 235.520	7.1.10	A	-	4	A.080	8	8	ŧ	8	T. T. T. A. A						
Spneumoniee 235.SEQ	T.T.T.	A T	•		A.000	8.0	8.0	•	8	. T. T. T. A T	•	į	5			
Spyogenes 235. SEQ	.1.1.10	A			A.000.	9.0.8	.0.0.1	ı	8		ŧ	į	8			
Mavium 235.520	C.TT. C.		:		8	8.0	8		8				5			
238.820	0.7	4			 8.c.	9.80	8		8	. c. 77	J		4			707
Ecoli 238.580	VACCORE	ACCOUNT.	MANOCO	CONTROC	TOTOCHA	COTTANT	COCCOCO	STOROGY	MUCCOCTA	ANACOCITICACORANGE CARACTER CENTROL ACTITIVATO CONTRACTOR CONTRACT	MAGGCOTA	CTATO.	CANACA			
				*******						2						
Minfluenzae 236.8EQ	A T.		:							100 C	4					
Sbrochiseptica 238, 539,	0			-	8				e	4	ŧ	Į				
Bpszapertussis 235.620					8	Ü	ú		e	4	ţ	ŧ				
Spartussis 238.580	0	-			8	,	Ü		c	0.00 C C C C C C C C C C C C C C C C C C	ŧ	ŧ				
Bospacia 235.520	0	•	,		8				c		ŧ	ŧ	,	,	;	
Emalle 238.SEC	90				8				o	.g		t	d			
Spreudomallei 235.52Q		•	,		8				e		f	ŧ				
Monorrhoese 238.550		4			ş		•			Lact D. Cont.	•	;	•	:		2
Meminjaititdis 238.830		4	ÿ		8	ú			c	•						
Paeruginosa 236.53Q	.A.A.C.A.	4			8		y	•	ŧ	.A.A.C.AA.						
Vcholerae 235.552	A . 7	:								26.1 26.1 3.13.0		4	5			1387
Venterocolitica 239.5BQ.AA.	AA	:	:	:	:	:						:	:			1404

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1410	420	1430	1440	1450	50	1460	1470	1480
Daubtille 215.520	ACC. CC. CACCATTT CA. A T T	A	.0	A O. G. AAG.	Ö	OTATT	A.A	204 S	8
Banthracis 238.520	ACC. C. TA. COTTTOM - A T. A	A T. A		awawa.	.T.	AT		CC. MOCA.	-74.00
Efacesiis 238.5EQ	A.TT. TOTTTAND. A T. A C. T A. GAATO A.T AT AA. G. AZ CC. AGCAA CA. TG. 1522	A E. A	T. D	A.CHATO.		77X		CC. MOCM.	5
Llactis 238.530	A.ACATGATC.TTA.	T2. A.	T	A. AGATG.		.A	AA.O.ATT.1	. O. CT. AGCA.	-QA.Q. 1507
Lmonocytogenes 235.5	monocytogenes 115.550AC. A.Tdrity-AACTTTTTAG.G-AATCAA.TAAA.GTGCCC.AAGA.			A G.G-AAT.	5	7. A	MA. OTO.	82.AOCA.	5
Saureue 238.520	ACC. A.A. COTTEMET		.TOT.	A G.COMO.		TA	X.0X	- CT AGCA	A.0C 1
Smutans 239.820	A.A	F A	.OO.	AC. A. NG.	1	XT	M. NO. NC	8. ADCL	-CA.G. 1504
Spneumoniae 218.820	A.A	F T. A.			Α.γ		M. AOT. 1	CT. AGCA.	0.45
Spyogenes 218.880	A.A	F T. A.	T0T.		A		M. NOT. C	OCT. AGCA.	9.0
Mavium 235.55Q	C.TOTA. 00000TCCTAAT. ATCA. C. T. INACCACCAA. ACC. A. CAACCAT. CCTTTCOO TOOCCAATTCOOCCT. COT. COAC. TTC. C. GOTACTA C. A A. 1636	7 MICA. C 7.	TACCACCAA.AC	A. COMOCATT.	CCCTTC00	TOOCONTTCOOCCT	.COT. COMC. TTC	C. COTACTA.	C.AA 1
Htuberculosis 238.820		ATCA.CT.	TANCCACCCAA.AC	A.OGA.CACT.	CCCTTCOO	T. TOCAGTICTOGGGC	. OST. OGU. TT.	C. GGTAGTA	C.A0.2
Zcoli 238.8EQ	TOSTOTELCT	CGANOCOCION	COMON	COCTATOTICOC	900	Ø	DOTTOTOCO	COTTANGOOD	1-100CT
Coneumoniae 238.580	1478			Α		***************************************		4	
dinfluences 238.830				T G MT. C		TT	A.A. OTO	P	2
Sbrochiseptica 235.8	bbrochissptica 115.88007007DC.0		DITQ	C.G. G. G.		TT		OCTOCAT	-CAGA
Sparapertussis 235.2	parapartussis 235.550 0TOTAC.0		a	C.G. G. CG.)		7		OCTOCAT	- CASA
Spertussis 238.5EQ	GTGTMC.0TFTCGC.GA.0CMF.ATTT		pr	2C.GA.0CQL.)		T		F OCTOCAT	-CMG
Bospacia 235.930	ATTOT. AA		p	Za. a		TT.		. O. COCTOCAT	100.0
Emalle 238.820	OTOTI: NAM		ď	X.G. 6		TT		.a. cocrocur	-G.AGA 1
Speeudomallsi 238.530			orTo	C.G. G 7.		TT		S.G. COCTOCAT	100
Mgonorrhoese 235.5E	Gonorabose 235.550A.TCA.A	2.3			J. W.	TT	T. DATAG. T.	8	101
Newinigititatis 238.4	Weningititdis 338.559. A.TCLA	2.5			VA.C	TT.	T. DATAG. T.	8	2
Pasruginosa 238.880	.CTGCTG	T. A		0.00		L	4	A	-
Vcholeres 235.820	.CTGAG. T				· · · · · · · · · · · · · · · · · · ·			0.1.0	-
Ventermonal Price 274 dBm									

יין מני טוני

Alignment Report of Gram +4-238 align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

Backsteis 218, 559
11
TAMANTO. T
MOGOTIO C
13.8 (1
Birochia ptica 135 stg1; ddct7;
7. 000077
Enails 135.500 A. 000007
PROPRIETE STATE STATE AND STATE STAT

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1	ŀ		ŀ		ŀ	ŀ	-	F	ŀ	-		
•	7	ec.	1300	25.0	1280	989.	9091	1610	1620	1630	1640	1650	
Baubtilis 238.820	ş	1	NOC*TT., TO*T., ACA		8	00.0.0	200	ŀ	1	0 10			1201
Banthracie 238.5EQ	8	TCTTTO.	MCC TCTTTG C G A	•	88	A. A. OC.	500		4	5	6	2	1308
Efacaelis 238.5EQ	ğ	10.70	TTC, TO T ACA G	-	910	W. A.	8	-	-	6	£	į	130
Llactis 238.820	į	57.70	.CTCT.101.ACA0A		OCCUPAN.	7.7	8			2.00	4	ų	1686
Leonocytogenes 218.8EQA.G TTC.TG.TT.TT.TACGT.AA.G.AAA.A.TGCCG.A.A.A.A.A.G.G.G.G.G.G.G.G.G	D.A.G.	110.13	TT. MC OT			AOT.GTA.T	8		4	5	£	8	1707
Saureus 238.880	8	TOOTE	CT. CO. : TOTATO. TT. ACA G GA		ant	M. T.00	8		4	TV Y	6		1702
Smitens 238.880	8	1.08	NGC 1. 074 1. 1. 0. 077 1. 1. 0. 077 1. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.			7.A.TMCT	8		4	8.8	9	U	1684
Spneumoniae 238.520	ğ	11.000	TT-070-C-1-ACA0 A	-	.1.1000	VC.19CT	8			Q. C	7	8	1688
Spyogenes 238.550	ğ	ě		-		UO. DACT	8			8.8	20.00		1690
Mavium 238.550	8	TOOM:	TTCGGTG . C. T d		88.0	-C.TMCA.O	8	Α					1871
Mtuberculosis 238.5EQ		TOOM:	.0CTT000T0 .C.T 0		88	0.000.0	8			·			1901
Ecold 235.5EQ	ğ	acucin	OCTANOCHONINTOCCTOCTTOCHOSTICTMOCHTCHOTANDATICHANICATHOCCANACOHOLOGICACHOTANOCHANGOCTTTANAAAATT	GUANACCE	CTANGGATCA	CTANCATCA	MTOTACC	CANCORC	Cognon	HOOTHONDAY	CCAACCC	THE PERSON NAMED IN	,
Koneumoniae 238.520	:											e	
Hinfluensee 235.SEQ	:	2		1	3	CT.	2	4			ŧ		788
Bhrochiseptics 235.580 00070. Troub0.0	800	.10.1100	M-0.0			-CTOT-CG	0.0		ð	D. AT. AT.			3
Bperperuseis 235.5E2000070.TJCDA00	200	.TO.TTOG	M-6.0			-0101.00	0.0		đ	TA TA D			
Spertuseie 238, SEO	ģ	.TO. TTOG	OCC70.TTOGAA0.0			5000	0.0		đ	A AT AT			9
Bospacie 238.820	ğ		AGCTTGGAAAGGATTCT. ACGATG .CGGQGa. ATT. T.			TCT, ACCUS	g.ca		d	£ 77.	£		1643
Smallet 235.650		1	AGCTTGGAA-G . GA			TCT. ACCUS	Ø.C			. CA. AT T.	£		1646
Spaeudomellet 238.880	Ξ.		COCTTGAAA~~QGA	:	•	TCT, ACCU	d.c		9	GA. AT T.			1646
Ngonorrhoese 238.820	8	B	CMCCOL.A.A.A.C	•		F. 75			9	T A.T	4.4		1658
Nemanigataldis 238.550 CAC	9	8	A. AC.	•		7.75			9		F.F.		1657
Paeruginose 238,820	ġ	ė	.AC100710	•		6			•				1648
Vcholerae 238.550	9	. T.	AGTC: 2TGA	:		A. GTCAG	:						1642
Yenterocolitica 218.SEQAT.GTTGA	5E0A.:										2		1660

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table.

Tuesday, November 27, 2001 4:14 PM

	ŀ	ŀ	-	ŀ	ŀ				ŀ	ŀ	l		l	
	1660	1670	1680	1690	1700	1710	1720	1730	1740	1750	1760		1770	
Baubtilis 238.520	ų	,	8		FC T	CHOS	906	***************************************	8	0.00	70.77	90000		1805
Banthracis 238,520			VQC.		T. T	3-6			9	3	70.77	9000C	c	1801
Efacaelis 238.8EQ	2		YOK.		175. T		- VCT	Y	20.00		24.25	3		1791
Liactis 238.5EQ	4	,			7C. TC		10.	***************************************	Ü	0	C 70. AT	300		1779
Emonocytogenes 235 SEGTC, .TC	OTCT		89			CTAT.	- 300	***************************************	8	0.0	C. 70.AT.	90000		1811
Saureus 236.SEQ	2		ğ		7C	3	1	***************************************	8	.20.07	C. 70.AT.	2000		1805
Smutens 238.580	2		8		TC. T	0	CCAT.		2		C. 70.A.	.0000	:	1776
Spneumoniae 235.520	2			:	TC. F	9	AC. T		5.7	9	C 70. MT.	.000C	:	1780
Spyogenes 238.520	2		8		FC. 7		AC.T.O-	***********	5		C TO . AT.	. OCCCA.	:	1781
Mavium 238.520			8		FT	.c.csxcc	5		0.0	7.0.0		5		1990
Mtuberculosis 215.550			8	:	FT	.C.CAA.C	ð		0.0	20.02	CA	5		2012
Reelf 238.8EQ	OCOTOMO	PARCENGOCA	AAATGGTGCC	TAKTTOO	осотамовийствоссими постосостийст тесовивальност вытиством пестововательствостанизательност постостосност посто	CTOATATOTA	ocrewone	cercoccana	MOCTON	ATCACTOC	MANTACC	OCTODOCTO	CAACTOTT	1777
Kpneumoniae 235.5EQ				:	1775	0.0							:	1775
Minfluenzas 235.8EQ	:	. !	A.G			.C.000.A-G	ATT. A00	OA000.	5.0.1	.0.0.	:		:	1783
Bhrochiseptics 218.5EGA.A	70 Y . Y	,	T. A.A.		£.1.	cc10	.TOTO	D.CTCOCT	DIA	88	CO.ATC			1755
Bpsrepertuseis 135,550A. A	0AA.		t A. A		£	cro	.TOTO:	0.01000	, ATG	98	C G.ATC			1756
Spertussia 238.820	A. A.		T. A. A.		A. A	cra:-	.TOTO:	0.010007	PY	8	C O. AER			1756
Bospacia 238.520						-0	0.76.0.0	8	900	8	C.AEA.A.3			1761
Bralle1 238.85Q	٠.٠			:		C16	.c.10.c.6	. S. C. T. C. S. D.	A 0070		C.AEA.A.7			1764
Speeudomailet 218:SEG A							.c. 78.c.e		A08	8	C.ATA.A.	1.0.		1764
Mgonorthoese 238.550			T A.A		T.T.	.ccrera.	Υ	ATT000T	8	2000	CO.M.			1775
Neminigititais 138.5222	Y			:	4.4.	0-cicis.	4	u	8	3000	C O. M.			1774
Pasruginosa 238.620			5			.c.9case	3	ITT. ACTICOD.A	Ē	30010		8		1765
Veholerae 238.550	:		4	:					:	SOM			:	1760
Venterocolitica 218.520		:		:			8	KT.TA.000.	:					1780

Alignment Report of Gram +&• 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	1780	1790	1800	1810	1820	1830	1840	1850	1860	1870	1880	1890	
Baubtilie 238.880	8	δ	CGOC.	78	9.0		•				188		1921
Banthracis 239.550	8	*	C60C.	T0CA	66.		•		.00	0.5	9		1917
Eracaelle 238.5EQ		6		T AT A	8	:	•		c	C-1100C.	AGA		1907
Labelte 4.48-82	8	ŧ		6 E	0 6		•			, i	0.7.0		1897
Saureus 238.SEQ	Ü	6		5	8		•			0-110	1		1927
Smutana 235.SEQ		•	C O MT.	T MT A.			•			AC TTT. T-C.	G.G.G.	ŀ	189
Spneumoniae 238.820			C	.T M					··· G········ T·· G··· T·· AT· T·· AT· T··· G·· G·· G·· ······ T······· T······ GA· GA· T·· C· T·· C·· T·· G·· G·· AdA· T·· ··· 1896	Q70	G.A.GA.		1896
appodents and and		• 1	1		0					9	3		1897
Memberrallosis 218 cm		5 8	5 6	200	2		•						3110
Zcoli 238.5ZQ	TATTAL	COCOCIC	CTCCAAACAC	CANADOCOCO	TATACTORE	A CONTRACTOR OF THE CONTRACTOR	T. COLOR	10000	SATISMAN CONTRACTOR CALL AND CALL AN	8			2132
Koneumoniae 238.520	:								4.5. T. 5. 4.4	C- 7 -0-1			188
Hinfluentee 238.820					9	•				TC 30-A			189
Bercchiseptica 235.520			C										1862
Sparapertuess 235.820	:	:											1863
Spertuests 235.5EQ								:		-			1863
Beallet 218.820												6	186
Bpseudomallet 235.582 .A	4		ú		o								187
Ngonorrhoese 235.8EQ									. A.A.C		ATG		188
Nemingiticis 235.820							•	:	A.AT		ATC0		188
Veholerae 215 SEO		:	5						1881	Ý.			1883
Tentercol. 11676 218 500	g						f		9,81				187
												:	Š

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue welght table. Tuesday, November 27, 2001 4:14 PM

	1900	1910		1920	1930	1940	1950	1960	1970	1980	- 661	2000	2010	
Baubtilis 215.630								0	4		8	9		2041
Efacacits 238.550	4	:	:			:			A7.		5			2037
Llactis 238.520										Å	5	.AG.		2027
LannonCytogenes 218.53Q.A	A 100 00 00 00 00 00 00 00 00 00 00 00 00	i						,	4 4	8	0 8	9 4		2017
Saureus 238,820		:						o	1	.A	Í	5 5		2047
Spneumeniae 238.820											ģ			2014
Spyogenes 215.520	A WINDOWS A CO. C. A WINDOWS A CO. C.							·		acam.	1	5 6		9 5
		2		1						70. Ch	1 3	, , ,		2230
Reolf 21s. sm	70	2		5						CCTT.71	3	.AA.	0.0	2252
08.820	ominated de la composition della composition del		MCIALAND.	ZICTIAN	OTACCIAN	rrectron	SOCTANOTE:	CONCETOCA	CONTROC	TANTANTOOCC	AGCTOTCT	cocceaac	reporter	2013
Hinfluensee 238.920	4									2017	:	:		2013
Bhrochiseptica 238.880	5075								•		:		:	2019
Bparapartussis 215.000.										:	5			1982
Spertussis 235.8EQ	100 11 11 11 10 10 10 10 10 10 10 10 10										o c			1983
Bospacia 238.830			:							Ü	đ	F	Ü	1988
38.820										1667 1.05	đ	•		1991
								,				•		1881
Neminigitetedis 238.580.A.								,		Ü	đ đ			2002
Paeruginose 238.620	1907			:						8	8		;	5 5
Fentencollisis 254 Fentencollisis 254	1986												:	1996
													:	2016

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table.
Tuesday, November 27, 2001 4:14 PM

	2020	2030	2040	2050	2060	2070	2080	2090	2100	2110	2120	21.10
Baubillis 238.530	. AT. G. A	١	6	7. A. G. C.		100	١	-				
Banchracis 235.520	AT.O.A		6	. AT.O. NC						; ;	•	
Sfaceslis 235.5EQ	4.0.1		5	2357 - 727 -		0	6					
Llactis 238.530	T.		6	7. C.		0.0	0		T COMPANY			
Amonocytogenes 218.820Ar.0.Ac	Q AT. G. R		6	λο		.0.0	2. A. SC. A. C	7.10	D ATOT OT		•	
Saureus 233.920			5	. COR. CO. AC		.0.0	0	ŀ	AT.C.OCA.P	ş	•	
Smtans 238.820	7.0°E		8	TT-0-AC		.AG.G	8	1.1	T. CTOTTAC	9	4	3
Spheumoniae 235.520	2		8	TT-G-AC		.A0.0.	8	11.1	OTO CTCTA.C	Ş	4	1
opyogense 235. SEC			8	TT-G-AC		.3.00.	8	T.T	OT. CHOTA.C	, VC VC.	4	4
Menharmilante 130 cm			1				.C C. A.	9.1.0	TOT.C.OTA.O			4
Member to the 438.50		CAA. A.			Α.		.C C.A.	6	TOT.C.OTA.O		***************************************	٠٠٠٠
Konsumoniae 216 cm			TATAL MINETAL	ALI MALLI MANAMININI MENDOCO COCHANIZA MANAMINI MENTANDA MANAMINI MANAMINI MOCETA ALIANI MANAMINI MANAMINI MAN	CHARACK	CONCENCE	TACTATACC	TOCCION	ACATTGAGCCT	TCATOTORA	XXXIXXXXXXXXX	ADOCTETOR
Kinfluennae 218 smo	1		,	100					?			
brochisentice 234 gp	90			C 2139				:	VI			
Barranettusis 215 send.								9	TGAG	88		8
Destrueis 238.500 0.0.077. 7. A.C. 7. 2103	0	ŀ		ŀ				P 1	2	8		8
Bospacia 235.830	0.00		٠ •	00.077						8		ġ :
Bralle1 238.520	0.00		0 4	00.0777			,				:	
Bpeeudomellet 218.520 0	0.00		Α	E		4						
Mgmorrhoes 215.530 G G	0.00		7	ŧ							:	
Naminigititdis 218.52000.0.T. AATOGTA.CA.CA.C	0000	Ę.	4	6						į		
Pasruginosa 238.830			f	A. 2121								Ψ
Vcholerae 215.630				2121			,					:
Yantarocolities 216 870	•								··········			

FIG. 2B-21

Alignment Report of Gram +2-23S elign.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:14 PM

	2140	2150	2160	2170	2180	2190	2200	2210	3330	2230	2240	2250
Bsubtilis 218.520	.XXX6.		ACC:MG	8	ATOTOO	8	000000		900	Y		١
Banthracis 238.650	. 300 340.	7	ACC A4 7 CT : 077		CACTOTA	*	00007	70,1.	CHOCOCO, CA.	4	Ü	22
Efacaelis 238.8EQ	CACCA.	.1	2264		41.015···	8	8	, Y. Y.	8	4.4	0	2
Llactis 238.5EQ	ABA.	T. T. T TAT	AZATTEXTTGTTGOGTGTA.G.T.ACCC.CTOG.A.AG.COAG.GAACACACAC.		AT. 0.0. TA	0.7 M	S.CT00	A.A	0,000,0A	Q	ą	33
Immnecytegens 135.5EQ auctr-10 T CA Acch G. A. ftcd	Jacon-110.	.T. C100.	Q. A. TOO.	8	OOCTOTA	alock	28.89	96.1.4	OC. TOO. CM	Α		
Saureus 218.520	ACCT AG	.1. (310)	AGGT-AGTCT AGGT G CTGG OG T AGCTGTG OCT	8	AOCTOTG	ğ	8.0		or	A		33
Smutans 238,820	AGTA	TT MC.T	. AGT A T T AG. T	8	4. of	.a.c.Ac	cc.,TMG	5	ATCTAC, GA.	,	,	33
Spneumoniae 215.520	G.10	3	GATC	8	970.TA	0.000	QQ		CTA.C.OA.	92		
Spyogenes 238.520	-	91	CTTC	8	OTO. TA	G.C.AC	£88	0.1.	CTA.C.B.	,		33
Mavium 238.880	5	T-01. T	.Ch-Ch		T. GATCOTA		CA.		Q. T. C.	00	•	7
Mtuberculosis 238.550		10.86-6			T. GATCOTA	28	C.CA.		G. T. MG.	00	1	77
Ecoli 238.SEQ	A0-TOTOGACO	CONTINUES.	Ad-TOTOLOCOLOTTICAT-GUACCOLOCTTOLARIAC LOCTTIVA SOTT CANCIDE LOCATICA ACTOR CONTINUE AND ACTOR A	CAMATACCAC	CCTTTANTOT	TOMOTHOR	NACCTOCNC	A-4000	COORTICOOC	Contractors	CONTRACTING	ACTOR 2250
Kpneumonfae 235.5EQ	Ÿ					:		Ų.	9			
Hinfluensee 235.520	Cuot	ATTG.	CAGT					ACCH.	5			2
Bbrochiseptice 118.880.CTCA.TATOMGATAT	.c4.3.	AT@A	AT.		1100	8	C.T.O	-	A.C.O.	ð	AC.	22
Bparapertuesis 135.550.CTCA.TATG40AT	3.CTC - A.T.	NT GMG	AT.		tio	8	C.T.9		A.C.Q	5	٠,٠	
Spertuesis 238.820	.c.c	ATGM	.CTCA, T, At GMG AT			8			A.C. 0	5	AC.	
Beepacia 238.8EQ		.1					C.T.G.		.0.0	ð	A. C.	
Bmallei 238.820	.ACCA.T.						C.T.G			5	A. C.	7
Spseudomallel 238.820			.ACCA							5	A. C.	
Ngonorzhoess 235.8EQChh	5	P	f T T.		corre. c.	9	8			55	A. C.	
Neminigititdis 235.550.CAA	D. ChA.				00TO		8				A. C.	
Paeruginosa 238.5EQ	Ÿ	10.0					TCT.03.		A.C.A			:
Vcholerae 238.550	NC.				Б		T.A					
Vantenooralities 238 Std - A Co	-	•					1					

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table.
 Tuesday, November 27, 2001 4:14 PM

	2260	2270	2280	2290	330	2310	2320	2330	23.40	23.50	2360	23.70	
Baubtilis 238.820	0 2 4 2 00 3 4 2 00 5 00 4 4 2 00 5 00 4 4 2 00 5 00 4 5 5 5 00 5 00	Ŗ	. O. O	8	440		000	1	8		Į.].	
Banthracis 235.550		-	CCA.	8	3	4	200	4	8	1	2		:
Efsceniis 218.5EQ			CC.A.	8		4	200	4	8	4	200		23
Llactis 235.550	0		C. T.A.		1	4	230	4 7 4 7		1	1		:
Lamnocytogenes 235,520			CC.A.	8	JOHN . AT	4	5	4	8	1	7		245
Saureus 238.530		Į,	C. T. A.	8	40		CATAG	4	8	1	1		:
Smutana 239,930	.d		CA.	8	5	4	TC. 130	T 4 T	8	1	5		1
Spneumoniae 238,650		9	CC. A.	8	. Cal	A	60.5	T. A. T.	8	4	1		2
Spyogenes 233.550			CC.A.	8	CATT	¥ ¥	90.5	T. A. T.	8	•	1		2
Havium 238.520	19			8	A A	ð	8	2	8	1	2.7	1	2
Atuberculosis 135.529	72		CC.A.	8	A A.	ð	8	T AT C	8	•	C. T. MG	3	26
Ecoli 238.520	GOOGICTCCTCTAAAATAACAAACAACAACAACAACTTOCTAATCCTCATACAACATAAATAA	NGNCINGO	амамасма	VACCITACOL	Arceroad	COCTOO	DATTOOKE	CONTROCOS	MACCOAG	TOACTOCIAGO	CONTRACTOR OF	CONCENT	2336
Appeamontee 238.520	, y			4									1
Hafluensae 235.820				4	ý	5		•	4	4	000		2
Bbrochiseptics 238.520			F	Ş	COLLAC	4	t		8			,	5
Sparapartuseis 236.529				Q	SCINC	4	t		ŧ	•		1	:
Spertuseis 238.820			H	, K	COTAC	4	t		ŧ	•	1	4	
Bospacia 238.550	C			S,C	COTAC	4	4		8	•	2		2
Brallei 218.820	234			, v	COTAC	7	7		6	4	3		2
Bpseudomailes 215.5EQ				, YC	COTAC	٠.٠	Y		5		S		ä
Ngonatrhosse 235.550			t	g		٠.٠	MCT.A	•	5		X	0.1	2
Nemingatituts 235.852			£	ý		AO.	KT.A.:.	4	5	······································	88	-	2
Paeruginosa 238.880			-	8		A0	7C.C60	AT	8	1	9		235
Vcholerae 238.520					AC7	5				γγ	5		2
Ventercolities 23s, SEQ				4	Q	5							
													i

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

	2	2380	2390	2400	2410.	2420	2430	2440	3450	3460	2470	2480	
Baubtilis 238.820	5	0.0		Ü					φ	1	ľ		
Banthracis 238.520	\$			U				·	2.0. Dit				
Bfacaells 238.820	5			Ü				r U		į		;	
Llactis 238.5EQ	£	A. T.		A.C.C.				e U	A	f			
Lacrocytogenes 238.382GdATC., C.T	, T	0.7		0.0.				U		į			25.2
Saureus 238,850		. A. T.		0.0				Ü	01.0	ŀ		,	
Smutane 235.520	3	0.7		A.C.T.				ů	GA	ť			240
Spnewoniae 238.5EQ	5			F. 0					O	0.00		ú	G.MG. 2492
Spyogenes 238.5%	5			A.C					GA7C0.T. TC0.T. T. CT.C				.0.30. 2493
NAV10m 238.8EQ	5	g		G000.0.1	3	TO. A.			GA55. GAG	Ė	•		CATO. 2705
House and and and	3	Ġ		GCCC.C.	,	2	:		φτοφ.σος		•		OATO. 2728
BOOK 230.000	Mediana	4	AND THE PERSON	ADDITION OF	TOCHACCO	CATCOCTCA	COCATANA	COTACTOCO	WOOMMACKATOKEKATALEEGAAGATTETAANTOGAAGOCCATCOCTCAADDERTAAAAGTACTCOGGGGTATAACAGGCTGATACCGCCCAACAG-TTCATATCGACGGCGTG	TGATACCGC	XMCG-1	TCATATOOK	0000000 2489
The Change of the Care	'	:					:	:	5878	:			
Threshippens of 120 cm					:			:	595		•		. !
Property and the second								•		:			2451
CSPS.						:					:		:
Bospecia 238.820				•			•		998		•		
Smallei 238.620				ı				•	2005				
Bpseudomallat 218.630				£-				•					
Ngonorrhoese 218.52Q	:			+						F			1
		4											4
Pagruginosa 238.880								:	. 2477		•		
Venoteree 238.880		•						:	2472				:
101101000111100 770 075 075 075 075 075 075 075 075 0								:	:				

25	2490 29	2500	2510	2520	2530	2540	2550	2560	2570	2580	8	2600	
Baubtilis 238.5EQ					Į,		2	ð				. 0	2637
Banthracis 238, SEQ .		. !			, C		2	ð	Ü				2632
Efacaelis 238.SEQ .							12	5		3			2621
Llactis 238.8EQ			0				p			3			2610
			0				2	ð	0.0	3			2642
Saureus 238.820					J		19	ð		,			2636
Smutana 238.820				g	9.4		P.	5		,			2609
Spneumoniae 238.520							10	ð		3			2611
22			0	g			2	5		y		.GGGGGGCCCC.	2612
Mavium 238.880			9		00	Α	10	ฮ				2834	2824
Mtuberculosis 235.5EQ .			9	9	0		2	đ				1, 2, 2,	2847
Scoll 235.520	TOOCIO	TOTALDI	COCCUCATO	эсктестора	CTGMOTAC	TOCCANOCO	TATOCCTAT	COCATITAN	AGTOGRACOC	CARCTOCOTTE:	NGAA-COTCOT	TTGGCCCTCAIGTGGGCCTCATCCTGGGGCTGAAGTAGTCCCAAGGGTATGGCTGTTCGCCATTTAAAGTGGTACGGAACTGGGTTAAA-CGTGGTGAGAAGAGTTCGGT	2608
Konemoniae 238.830													2608
Hinfluentee 238.822			:	•		:		:					2614
Abrochiseptica 238.88g					8							F	2569
Sparapertusate 238.582				4							k		. 2571
Spertussis 235.520					8			4				T	. 2570
Bcepacia 238.820		:	:	£	8						.A		. 2583
Brallei 218.8EQ			:		8						A		. 2586
Spseudomalle 218.550							:::::::::::::::::::::::::::::::::::::::				.A	•	. 2586
Mgcnorzhossa 218.5EQ			:								A.1-		. 2597
Neminigititdis 238.570					0				-		A		. 2596
Paeruginosa 238,5EQ					8							5986	. 2596
Vcholerae 235.SEG		:	:									2891	. 2591
Vantamond (tips 316 Sto													

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

		ŀ										I	
	2610	3620	2630	. 9.	2650	2660	2670	2680	2690	2700	2710	2720	
Baubtilis 235.820		ť	C. C. C. A. T. C. T. C.	-A. A.	5.7		3	0.0	D.4	ų	9	p	275
Banthracis 238, 820	9			AA	5.5		ð	500	2	ų	44	2	TG 2750
Efacaelis 238.520		0		AA	70.7		8	T.C. 0.	5	Ę		2	.70 2739
Llactis 238.5EQ		7		AAE	7.7		8	TT.C.G	A.G.	2	100		70.2728
Innoncytogenss 238.880		0	A. T.	AA.	70.7		ð	A. C.O.	5.4	ğ	12	2	70 2760
Saureus 238.530				AA	7.2		ð	AT C.T.	5	0	¥2	p	70 2754
Smutans 238,820		9	G.T. G AA A.TTA AT	A AT.			A	10.0.1	3	ų	5	2	0 2727
Spneumoniae 238.820		9		AAT			Α	T. C.O.	5.4	2	γΥ	2	6272
Spyogenes 238.520			C.T. CTTT	AAT			Α	T.C.G.	24		77	p	70 2730
Mavium 235.820		ن.			'n		3	A.C. T.	5	8	8	P	C.00 2942
Htuberculoeis 238.520			.*	MMC	2		a	A.C.T.	5	8	8	P	2962
Ecoli 235.5EQ	CCCTATCT	SOCIAL	OCTANTIOCOTOGGGGTGGAAGTGAGggggGTGCTCCTAGTAGAAGACGGGAGGGACGACACAGAGTGTGGGGTTGTGAGCCAATGGGACATGCCGAGAAGTAA 2126	WOGOODCT	CTCCTAG	TACCACACIOCAL	прополого	COCHECACT	promore	GOLDAGO	WIGOCOCT	GCCCGGTABC	TAM 2726
Kpneumoniae 235.520	:		200 P										2726
Kinfluensse 235.820		٠	Th. TG.T. T-T.							g	.TT.		2732
Bbrochiseptics 238.620	:			ACA A.C				7.0.7	A.C		-		0 2689
Bpsrapsrtussis 236.620	:		15.1.1	. ACA: .A.C.	:			10.5	k .c.			0	0 2691
Bpertussis 238.550	:			ACA A.C.	:						-	o	0 2690
Bospacis 238.520					-			A.C.T	A.C.	3	5	0	10 2701
Brallei 235.550		•			:			A.C.T		0.0	.a.c7c		2704
Bpseudomailst 238.650			TAOFT.		:::::::::::::::::::::::::::::::::::::::			A.C.T	A.C		.o.c7c	0	70 2704
Ngonorrhoese 238.882	:	4			:		•	A.C.T	A.C	A.C.	4770	0	G 2713
Neministitudis 238.880	:	4	T	-	:			A.C.T	A.C		ATTA		0 2714
Paeruginosa 238.530	:	:	A. T.	A	:			A.C.T					70 2714
Vcholerae 215.580	:		7		:			A.C.T.		9	C. CO		2709
Yanterocolitica 238.820 Y XI.YR	Q		Y X.					М.			>		2720

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

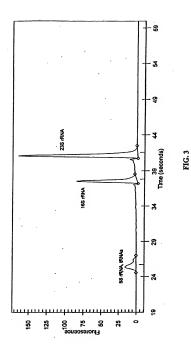
		-	l										i
	2730	2740	2750	2760	27.0	2780	2790	3800	2810	2820	2830	2840	
Baubtilis 238.830	0.0				300 T. A.	λ.Τ.	ATT. OC	-A.0.MOT	ATC. C	5	. C. G	2 7	2871
Banthracis 238.820		:			000.T.A.A.	A.T	.AT.0.0.	-A. OCTAOT	ATC.C	5		710.70	2866
Efectelis 238.630		×			83. T.A.	A.T	.T.TT.T.	.A. GAMOT		.000	AG6	1	2656
	36				88.7.7.	A. 7A.	ATT.Q	3. A. GA-A. T	2000	ADMG T.	36	2	2842
238.580		Ş		6	887.A.		.Y.T.T.	-convior-	XEC.C	3	TCG	. T.	2876
Sauceus 238.880		:		-	8	A.T.	AA.T.	-000TTA-T-	ATC.C	5		TTC.AG	2867
Spreumoniae 238.630	, o	9		101			. AT. A.G.	C.OTTAGE	900	4 6	Add G. M.C Add Add A. M.	£	2844
Spyogenes 238.820	, D	Ü		2	8	•	ATCASE	77.70		1	AG . G		
Mavium 238.8EQ	T. C.O.	8		8	4 7 7 3	0		100	٤	,	20		
Mcuberculosie 238.550	.1100	8		8	C.T.T. A.			100	8	9	7. TO 00. C. C. T. T. A. C. G. T. T. A. C. G. T. T. A. C. C. C. T. T. A. C. T. T. A. L. C.		200
	TOCOGNAGA	TAMOROCTO	MACATOTA	MOCKERNIC	TTGGGGGA	GATGAGTICTO	CTCACCCT	TAMOORICCE	SUCCESSOR	TTGANGAGO	тесквиминия и постаминения пост	goodganar	2846
	:						3				23 986		2846
Hinfluenses 238.650						5		NO.	9			t	2848
Ebrochisaptica 218.880. A		8		8	C.T. TO.	T G. A.	oocic		5	8			2808
Eparapartussis 238.822.A		8		8	.C.T.7GA		7-20-T	2.AG.TCCC	8	8			2808
Spartuasia 235, SEQ		8		8	.c. 7. 704	T. O.A	8-K		8	8	.h		2807
		8		8		A.A	000	. AG. TOCC. T.	5	8	T		2821
BERTTO 738 BE		8		8		, Ç	.c.00GW	d. TCCC. T.	8	8	T		2824
EDB CONTROL 218.582 TT		8		8		NCT.		6.TOCC.T.	6	8			2824
Design and the state of the sta					1		8	8	5	8		2	2833
3		: 1				, ve	8	5	5	8		2	2834
3		į		8	T. Y.	A	¥	0.1100	8		TAAAAAAA.		2834
Venoterae 438.880		: ا		0	5	t	ν	6	A9	8	. 2		2829
Yentstocolities 21S.SZZXSYRSRSRSRS		2		RS	, x		5		:			χ.	2849

Alignment Report of Gram +&- 23S align.MEG, using Clustal method with Weighted residue weight table. Tuesday, November 27, 2001 4:15 PM

	2850 2	2860 28	2870	2880	2890	2900		
Baubtilis 238.522	.07.70. A.CA.A.G	CA. A. G.	6.4.4	8	407			
Banthracis 238.650	.0ATG.T., CAT0		.o.		TNG.A.	A-T		
Efacaelis 238.830	.GGCTTACT.GGGAACCCTAG.A.	or.o.	8	88	T AG.A.	ν-γν-		
Clactis 238.820	.GA.TTGA.T.GGGACGCTAG.ATA-A	G. T.O.	8	8	T A.O.A.	.TA-A		
Lannocytogenes 235.5EQ.GT.TA.A.GCGANACG.TAG.AA-A	G. 7.7	A.A.O.	80. MA	8	TMG.A.	A-4-1		
Saureus 235.650	.0ATG.T., Chr0	0.50	.oak	. 8	T A A	T.A-A		
Smutans 238.850	.0TT.TChChCoNhCCTNG.A.T.T.TA-A	5	3.18	8	T26.A.	7- Y-Y	•	
Spneumoniae 238.520	.0T.TGCA.A.OTQQTMAMCTMG.ATA-A	CA.A.OT.	77.	ă	T NO. A.	4-4-5	•	
Spyogenes 238.820	T.T A.A.OT 00. TAAACT AG.AT.	. A. A. OT	22.50	Ş	TNG. A.	E		
Mavium 238.880	.6TTAAQCQGTCCTQQA.A.	D		000	-4.4			
238.850	.6TTh6OTO3T6C000	000		9	- A. A.	MCA		
Ecoli 238.5EQ	OTANGOSCIA CONTROLOCTIA COSTACTA A TOTA A COSTACA A CONTRA A CONTR	ATGCCTTCACC	TACCOOK	ACTANTON	COSTOROGE	PAGE TO		*
pneumoniae 238.820								
Hinfluenzae 238.650	T.M.T.OT.A.	Q. y.	4	700	4			
shrochiseptica 238.580.0TAAA	.a		1	ě				
Sparapertussis 238.820.0	.OT		4	2		0.1.0		
percussis 236.SEQ	.0	Α	4	2		0.1.0		
•	ThCT.ATCCA		T.	2	Α	.0.1.0.		
mallel 235.8EQ			4	8	, ye	.0.T.C.A		
Spacudomalled 215.SEQ	42		4	8	30	6.7.C.A		
Ngonozzhoese 218.820 . GG.ZA.CGACATCCGTC.	.g	CG	5	200		.aTC.		
Neminigititels 238.820.00.73.CGACATGCTG.TC.	£.00.	.cg	5	2		.aTC.		
Paeruginosa 235.5EQ			4	P		.6		
Vcholerse 218.530			•	302				
Yenterocolitica 215.920	1000	1						

Decoration 'Decoration #1': Mide (se '.') residues that match Ecoli 218.8EQ exactly.

10 00 01



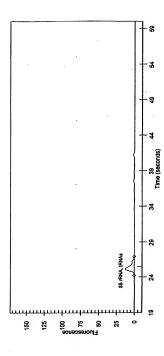


FIG. 4

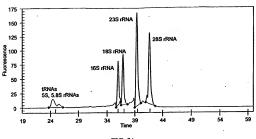


FIG. 5A

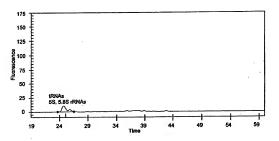


FIG. 5B

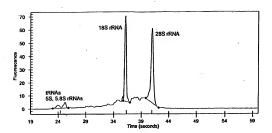


FIG. 6A

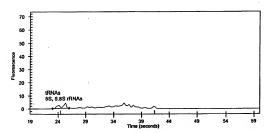


FIG. 6B

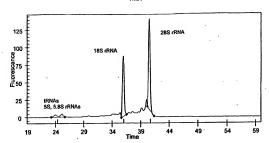


FIG. 7A

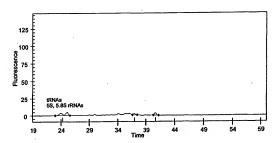


FIG. 7B

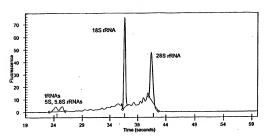


FIG. 8A

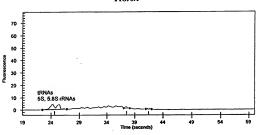


FIG. 8B

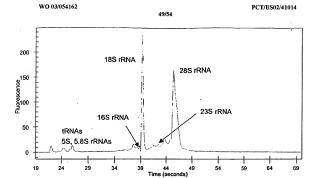


FIG. 9A

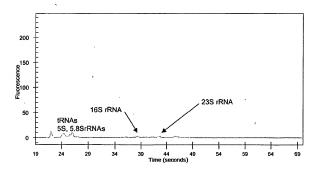
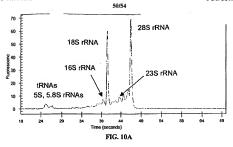
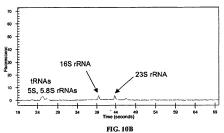
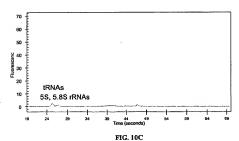


FIG. 9B

WO 03/054162 PCT/US02/41014







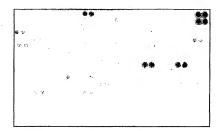


FIG. 11A

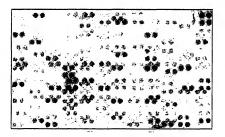


FIG. 11B

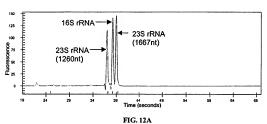


FIG. 12A

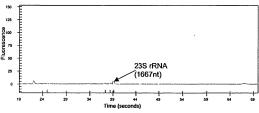


FIG. 12B

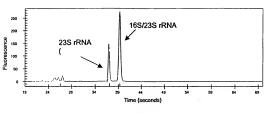


FIG. 13A

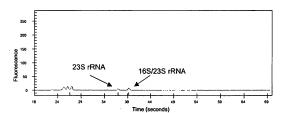


FIG. 13B

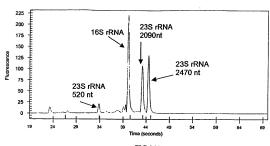


FIG 14A.

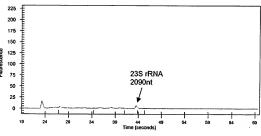


FIG 14B.

SEQUENCE LISTING

	x110> MURPHY, GEORGE L. WHITLEY, J. PENN	
5	120> METHOD AND SYSTEM FOR DEPLETING TRNA POPULATIONS	
	:130> AMBI:076WO	
10	:140> UNKNOWN :141> 2002-12-20	
15	(150> 10/029,397 (151> 2001-12-20	
	×160> 92	
	<pre><170> PatentIn Ver. 2.1</pre>	
20	2210> 1 2211> 22	
	2212> DNA	
	213> Artificial Sequence	
25	<220>	
	223> Description of Artificial Sequence: Synthetic Primer	
	:400> 1	
30	etgetgeete eegtaggagt et	22
	×210> 2	
35	211> 23 212> DNA	
33	2213> Artificial Sequence	
	220>	
40	<223> Description of Artificial Sequence: Synthetic Primer	
	:400> 2 :gtattaccg cggctgctgg cac	23
46	300000000 0330030035 000	
45	(210> 3	
	2211> 23	
	212> DNA	
50	213> Artificial Sequence	
	220>	
	223> Description of Artificial Sequence: Synthetic Primer	
55	:400> 3 :gcccagtaa ttccgattaa cgc	23

	<210> 4	
5	<211> 23 <212> DNA	
3	<213> Artificial Sequence	
	22137 Artificial Sequence	
	<220>	
	<223> Description of Artificial Sequence: Synthetic	
10	Primer	
	<400> 4	
	tggactacca gggtatctaa tcc	23
15		
	<210> 5	
	<211> 23	
	<212> DNA	
20	<213> Artificial Sequence	
20	<220>	
	<223> Description of Artificial Sequence: Synthetic	
	Primer	
	* T *	
25	<400> 5	
	gggttgcgct cgttgcggga ctt	23

	<210> 6	
30	<211> 23	
	<212> DNA	
	<213> Artificial Sequence	
	<220>	
35	<220> <223> Description of Artificial Sequence: Synthetic	
,,	Primer	
	FIIMGI	
	<400> 6	
	taaggaggtg atccaaccgc agg	23
40	55 00 0 0	
	<210> 7	
	<211> 23	
	<212> DNA	
45	<213> Artificial Sequence	
	<220> <223> Description of Artificial Sequence: Synthetic	
	Primer	
50	*******	
,,	<400> 7	
	ggttcttttt cactcccctc gcc	23
55	<210> 8	
	<211> 23	

	<212> DNA	
	<213> Artificial Sequence	
5	<pre><220> <223> Description of Artificial Sequence: Synthetic</pre>	
3	Primer Primer	
	<400> 8	
10	gacccattat acaaaaggta cgc	23
	<210> 9 <211> 23	
1.5	<212> DNA	
15	<213> Artificial Sequence	
	<pre><220> <223> Description of Artificial Sequence: Synthetic</pre>	
	Primer	
20	<400> 9	
	gcccgttac atcttccgcg cag	23
25	<210> 10	
	<211> 23 <212> DNA	
	<213> Artificial Sequence	
30	<220>	
	<223> Description of Artificial Sequence: Synthetic Primer	
	Primer	
35	<400> 10 cgacaaggaa tttcgctacc tta	23
33	cgacaaggaa tttcgctace tta	23
	<210> 11	
40	<211> 22	
40	<212> DNA <213> Artificial Sequence	
	<pre><220> <223> Description of Artificial Sequence: Synthetic</pre>	
45	Primer	
	<400> 11	
	cttacccgac aaggaatttc gc	22
50		
	<210> 12 <211> 23	
	<212> DNA	
55	<213> Artificial Sequence	
-	<220>	

	<223> Description of Artificial Sequence: Synthesis Primer	netic
	<400> 12	
5	gagccgacat cgaggtgcca aac	23
	222	
	<210> 13 <211> 21	
10	<212> DNA	
	<213> Artificial Sequence	
	<220>	
15	<223> Description of Artificial Sequence: Synthesis Primer	ietic
	<400> 13	
	ggttaagcct cacggttcat t	21
20		
	<210> 14	
	<211> 14 <212> DNA	
	<213> Artificial Sequence	
25	abas incorrect sequence	
	<220>	
	<223> Description of Artificial Sequence: Synth Primer	netic
30	<400> 14	
	ggaagcgcac ggca	14
	<210> 15	
35		
-	<212> DNA	
	<213> Artificial Sequence	
	<220>	
40	<223> Description of Artificial Sequence: Synth Primer	netic
	<400> 15	23
45	ccccttctcc cgaagttacg ggg	23
	<210> 16	
	<211> 21 .	
	<212> DNA	
50	<213> Artificial Sequence	
	<220>	
	<223> Description of Artificial Sequence: Synth	netic
	Primer	
55	<400> 16	

	gtgagctatt acgctttctt t		21
	<210> 17		
5	<211> 23		
	<212> DNA		
	<213> Artificial Sequence		
	<220>		
10	<223> Description of Artificial Sequence: Primer	Synthetic	
	<400> 17		
15	taccggccgt gcgtacttag aca		23
	<210> 18		
	<211> 23		
	<212> DNA		
20	<213> Artificial Sequence		
	<220>		
	<223> Description of Artificial Sequence:	Synthetic	
25	Primer		
	<400> 18		
	tgccctccaa tggatcctcg tta		23
30	<210> 19		
	<211> 23		
	<212> DNA		
	<213> Artificial Sequence		
35	<220>		
	<223> Description of Artificial Sequence: Primer	Synthetic	
	<400> 19		
10	Ctacggaaac Cttgttacga ctt		23
	<210> 20		
	<211> 23		
15	<212> DNA		
	<213> Artificial Sequence		
	<220>		
0	<223> Description of Artificial Sequence: Primer	Synthetic	
	<400> 20		
	gagcactggg cagaaatcac atc		23
5			
	<210> 21		

```
<211> 23
     <212> DNA
     <213> Artificial Sequence
    <220>
     <223> Description of Artificial Sequence: Synthetic
    <400> 21
10
    gtttetttte etcegetgae taa
                                                                       23
     <210> 22
    <211> 23
15
    <212> DNA
     <213> Artificial Sequence
     <223> Description of Artificial Sequence: Synthetic
20
           Primer
     <400> 22
     tecteageca ageacataca eca
                                                                       23
25
     <210> 23
     <211> 1427
     -212 DNA
     <213> Bacillus subtilis
30
    <220>
     <221> modified base
     <222> (554)..(873)
     <223> N = A, C, G or T/U
35
     <400> 23
    gagagtttga teetggetea ggaegaaege tggeggegtg cetaatacat geaagtegag 60
     cggacagatg ggagcttgct ccctgatgtt agcggcggac gggtgagtaa cacgtgggta 120
    acctgcctgt aagactggga taactccggg aaaccggggc taataccgga tggttgtttg 180
40
    aaccgcatgg ttcaaacata aaaggtggct tcggctacca cttacagatg gacccgcggc 240
    gcattagcta gttggtgagg taacggctca ccaaggcaac gatgcgtagc cgacctgaga 300
    gggtgatcgg ccacactggg actgagacac ggcccagact cctacgggag gcagcagtag 360
     ggaatettee geaatggaeg aaagtetgae ggagcaaege egegtgagtg atgaaggttt 420
     toggatogta aagototgtt gttagggaag aacaagtaco gttogaatag ggoggtacot 480
    tgacggtacc taaccagaaa gccacggcta actacgtgcc agcagccgcg gtaatacgta 540
     qqtqqcaaqc qttntccqqa attattqqqc gtaaaqqqct cqcaqqcqqt ttcttaagtc 600
     tqatqtqaaa qccccqqct caaccqqqqa qqqtcattqq aaactqqqqa acttqaqtqc 660
     aqaaqaqqaq aqtqqaattc cacgtgtngc ggtgaaatgc gtagagatgt ggaggaacac 720
     caqtqqcqaa qqcqactctc tggtctgtaa ctgacgctga ggagcgaaag cgtggggagc 780
    gaacaggatt agataccetg gtagtccacg ccgtaaacga tgagtgctaa gtgttagggg 840
    gtttccgccc cttagtgctg cagtaacgca ttnagcactc cgcctgggga gtacggtcgc 900
     aagactgaaa ctcaaaggaa ttgacggggg ccgcacaagc ggtggagcat gtggtttaat 960
     tcgaagcaac gcgaagaacc ttaccaggtc ttgacatcct ctgacaatcc tagagatagg 1020
     acqtcttcqq qqqcaqaqtq acaqqtqqtq catqqttqtc qtcaqctcqt qtcqtgaqat 1080
55
    gttqqqttaa qtcccqcaac qaqcqcaacc ctqqatctta qttqccaqca ttcaqttqqq 1140
     cactctaagg tgactgccgg tgacaaaccg gaggaaggtg gggatgacgt caaatcatca 1200
```

tgccccttat gacctgggct acacacgtgc tacaatggac agaacaaagg gcagcgaaac 1260 cgcgaggtta agccaatccc acaaatctgt tctcagttcg gatcgcagtc tgcaactcga 1320 ctgcgtgaag ctggaatcgc tagtaatcgc ggatcagcat gccgcggtga atacgttccc 1380 gggccttgta cacaccgccc gtcacaccac gagagtttgt aacaccc <210> 24 <211> 1544 <212> DNA 10 <213> Bacillus anthracis <400> 24 gtttgatcct ggctcaggat gaacgctggc ggcgtgccta atacatgcaa gtcgagcgaa 60 tqqattaaqa qcttqctctt atqaaqttaq cqqcqqacqq qtqaqtaaca cqtqqqtaac 120 ctgcccataa qactgggata actccgggata acattttgaa 180 ccgcatggtt cgaaattgaa aggcggcttc ggctgtcact tatggatgga cccgcgtcgc 240 attagctagt tggtgaggta acggctcacc aaggcaacga tgcgtagccg acctgagagg 300 qtgatcggcc acactgggac tgagacacgg cccagactcc tacgggaggc agcagtaggg 360 aatottoogo aatggacgaa agtotgacgg agcaacgcog ogtgagtgat gaaggottto 420 20 gggtcgtaaa actctgttgt tagggaagaa caagtgctag ttgaataagc tggcaccttg 480 acggtaccta accagaaagc cacggctaac tacgtgccag cagccgcggt aatacgtagg 540 tggcaagcgt tatccggaat tattgggcgt aaagcgcgcg caggtggttt cttaagtctg 600 atgtgaaagc ccacggctca accgtggagg gtcattggaa actgggagac ttgagtgcag 660 aagaggaaag tggaattcca tgtgtagcgg tgaaatgcgt agagatatgg aggaacacca 720 25 qtqqcqaaqq cqactttctq gtctqtaact gacactqagg cgcgaaagcg tggggagcaa 780 acaqqattag ataccetggt agtecaegee gtaaacgatg agtgctaagt gttagagggt 840 ttccgccctt tagtgctgaa gttaacgcat taagcactcc gcctggggag tacggccgca 900 aggctgaaac tcaaaggaat tgacgggggc ccgcacaagc ggtggagcat gtggtttaat 960 togaagcaac gogaagaacc ttaccaggto ttgacatoot otgacaaccc tagagatagg 1020 gcttctcctt cgggagcaga gtgacaggtg gtgcatggtt gtcgtcagct cgtgtcgtga 1080 gatgttgggt taagtcccgc aacgagcgca acccttgatc ttagttgcca tcattaagtt 1140 gggcactcta aggtgactgc cggtgacaaa ccggaggaag gtggggatga cgtcaaatca 1200 tcatgccct tatgacctgg gctacacacg tgctacaatg gacggtacaa agagctgcaa 1260 gaccgcgagg tggagctaat ctcataaaac cgttctcagt tcggattgta ggctgcaact 1320 35 cgcctacatg aagctggaat cgctagtaat cgcggatcag catgccgcgg tgaatacgtt 1380 cccgggcctt gtacacaccg cccgtcacac cacgagagtt tgtaacaccc gaagtcggtg 1440 gggtaacett tttggageca geegeetaag gtgggacaga tgattggggt gaagtegtaa 1500 caaggtagec gtateggaag gtgeggetgg ateaceteet teet 40 <210> 25 <211> 1449 <212> DNA <213> Enterococcus faecalis 45 <400> 25 cgaacgctgg cggcgtgcct aatacatgca agtcgaacgc ttctttcctc ccgagtgctt 60 gcactcaatt ggaaagagga gtggcggacg ggtgagtaac acgtgggtaa cctacccatc 120 agagggggat aacacttgga aacaggtgct aataccgcat aacagtttat gccgcatggc 180 50 ataagagtga aaggcgcttt cgggtgtcgc tgatggatgg acccgcggtg cattagctag 240 ttggtgaggt aacggctcac caaggccacg atgcatagcc gacctgagag ggtgatcggc 300 cacactggga ctgagacacg gcccagactc ctacgggagg cagcagtagg gaatcttcgg 360 caatqqacqa aagtctgacc gagcaacgcc gcgtgagtga agaaggtttt cggatcgtaa 420 aactotqttq ttagagaaga acaaggacgt tagtaactga acgtcccctg acggtatcta 480

accagaaage cacggetaac tacgtgccag cageegegt aataegtagg tggcaagegt 540 tqteeggatt tattgggegt aaagegageg caggeggttt ettaagtetg atgtgaaage 600 WO 03/054162 PCT/US02/41014 8/52

```
ccccggctca accggggagg gtcattggaa actgggagac ttgagtgcag aagaggagag 660
    tggaattcca tgtqtagcgg tgaaatgcqt agatatatgg aggaacacca qtggcqaaqg 720
    cggctctctg gtctgtaact gacgctgagg ctcgaaagcg tggggagcaa acaggattag 780
    ataccetggt agtecacgee gtaaacgatg agtgctaagt gttqqaqqqt ttccqccctt 840
    cagtgctgca gcaaacgcat taagcactcc gcctggggag tacgaccgca aggttgaaac 900
    tcaaaggaat tgacggggc ccgcacaagc ggtggagcat gtggtttaat tcgaagcaac 960
    gcgaagaacc ttaccaggtc ttgacatcct ttgaccactc tagagataga gctttccctt 1020
    cggggacaaa gtgacaggtg gtgcatggtt gtcgtcagct cgtgtcgtga gatgttgggt 1080
    taagtcccgc aacgagcgca accettattg ttagttgcca tcatttagtt gggcactcta 1140
10 gegagactge eggtgacaaa ceggaggaag gtggggatga egtcaaatca teatgeceet 1200
    tatqacctgq gctacacacg tgctacaatq ggaagtacaa cqaqtcgcta qaccqcqaqq 1260
    teatgeaaat etettaaage tteteteagt teggattgea qgetqeaact eqeetqeatg 1320
    aagccggaat cgctagtaat cgcggatcag cacgccgcgg tgaatacgtt cccgggcctt 1380
    gtacacaccg cccgtcacac cacgagagtt tgtaacaccc gaagtcggtg aggtaacctt 1440
15
    tttggagcc
    <210> 26
    <211> 1548
20 <212> DNA
    <213> Lactococcus lactis
    <400> 26
    tttatttgag agtttgatcc tggctcagga cgaacgctgg cggcgtgcct aatacatgca 60
25 agttgagege tgaaggttgg tacttgtace gactggatga geagegaacg ggtgagtaac 120
    gcgtggggaa tctgcctttg agcgggggac aacatttgga aacgaatgct aataccgcat 180
    aaaaacttta aacacaaqtt ttaaqtttqa aaqatqcaat tqcatcactc aaaqatqatc 240
    ccgcgttgta ttagctagtt ggtgaggtaa aggctcacca aggcgatgat acatagccga 300
    cctgagaggg tgatcggcca cattgggact gagacacggc ccaaactcct acgggaggca 360
30 qcaqtaggga atcttcggca atggacgaaa gtctgaccga gcaacgccgc gtgagtgaag 420
    aaggtttttcg gatcgtaaaa ctctgttggt agagaagaac gttggtgaga gtggaaagct 480
    catcaagtga cggtaactac ccagaaaggg acggctaact acgtgccagc agccgcggta 540
    atacgtaggt cccgagcgtt gtccggattt attgggcgta aagcgagcgc aggtggttta 600
    ttaagtetgg tgtaaaagge agtggeteaa ceattgtatg cattggaaac tggtagaett 660
    gagtgcagga gaggagagtg gaattccatg tgtagcggtg aaatgcgtag atatatggag 720
    gaacaceggt ggcgaaagcg gctctctggc ctgtaactga cactgaggct cgaaagcgtg 780
    gggagcaaac aggattagat accetggtag tecaegeegt aaacgatgag tgetagatgt 840
    agggagetat aagttetetg tategeaget aacgeaataa geacteegee tggggagtae 900
    gaccgcaagg ttgaaactca aaggaattga cgggggcccg cacaagcggt ggagcatgtg 960
    qtttaattcq aaqcaacqcq aaqaacctta ccaqqtcttq acatactcqt qctattccta 1020
    gaqataqqaa qttccttcqq qacacqqqat acaqqtqqtq catqqttqtc qtcaqctcqt 1080
    gtcqtqaqat qttqqqttaa qtcccqcaac gaqcqcaacc cctattqtta qttqccatca 1140
    ttaagttggg cactctaacg agactgccgg tgataaaccg gaggaaggtg gggatgacgt 1200
    caaatcatca tgccccttat gacctgggct acacacgtgc tacaatggat ggtacaacga 1260
    gtcgcgagac agtgatgttt agctaatctc ttaaaaccat tctcagttcg gattgtaggc 1320
    tgcaactege ctacatgaag teggaatege tagtaatege ggateageae geegeggtga 1380
    atacqttccc gggccttgta cacaccgccc gtcacaccac gggagttggg agtacccgaa 1440
    gtaggttgcc taaccgcaag gagggcgctt cctaaggtaa gaccgatgac tggggtgaag 1500
    tcgtaacaag gtagccgtat cggaaggtgc ggctggatca cctccttt
50
    <210> 27
```

<211> 1524 <212> DNA

55 <213> Listeria monocytogenes

```
<400> 27
     gcctgcaggt cgacaacaga gtttgatcat ggctcaggac gaacgctggc ggcgtgccta 60
     atacatqcaa qtcqaacqaa cqqaqqaaqa qcttqctctt ccaaaqttaq tqqcqqacqq 120
     qtqaqtaaca cqtqqqcaac ctqcctqtaa gttqqqqata actccqqqaa accqqqqcta 180
     ataccqaatg ataaagtgtg gcgcatgcca cgcttttgaa agatggtttc ggctatcqct 240
     tacagatggg cccgcggtgc attagctagt tggtagggta atggcctacc aaggcaacga 300
     tgcatagccg acctgagagg gtgatcggcc acactgggac tgagacacgg cccagactcc 360
     tacqqqaqqc aqcaqtaggg aatcttccgc aatggacgaa agtctgacgg agcaacgccg 420
     cgtgtatgaa gaaggttttc ggatcgtaaa gtactgttgt tagagaagaa caaggataag 480
10
    aqtaactqct tqtcccttqa cqqtatctaa ccaqaaaqcc acqqctaact acqtqccaqc 540
     agccqcqqta atacqtaqqt qqcaaqcqtt qtccqqattt attqqqcqta aagcqcqcc 600
     aggcqqtctt ttaaqtctqa tqtqaaaqcc cccqqcttaa ccqqqqaqqq tcattqqaaa 660
     ctqqaaqact qqaqtqcaqa aqaqqaqaqt qqaattccac qtqtaqcqqt qaaatqcqta 720
     gatatqtqqa qqaacaccaq tqqcqaaqqc gactctctqq tctqtaactq acqctqaqqc 780
    gcgaaagcgt ggggagcaaa caggattaga taccctggta gtccacgccg taaacgatga 840
     gtgctaagtg ttagggggtt tccgccctt agtgctgcag ctaacgcatt aagcactctg 900
     cctggggagt acgaccgcaa ggttgaaact caaaggaatt gacgggggcc cgcacaagcg 960
     tggagcatgt ggtttaattc gaagcaacgc gaagaacctt accaggtett gacateettt 1020
    gaccactctg gagacagagc tttcccttcg ggacaaagtg acaggtggtg catggttgtc 1080
20
    gtcagctcgt gtcgtgagat gttgggttaa gtcccgcaac gagcgcaacc cttgatttta 1140
    gttqccaqca tttagttqqq cactctaaaq tqactqccqq tgcaaqccqa qqaaqqtqqq 1200
    gatgacgtca aatcatcatg ccccttatga cctgggctac acacgtgcta caatggatag 1260
    tacaaagggt cgcgaagccg cgaggtggag ctaatcccat aaaactattc tcagttcgga 1320
    ttgtaggctg caactcgcct acatgaagcc ggaatcgcta gtaatcgtgg atcagcatgc 1380
25
    cacggtgagt acgttcccgg gccttgtaca caccgcccgt cacaccacga gagtttgtaa 1440
    caccegaagt cggtagggta acctttatgg agccagcege cgaaggtggg acagataatt 1500
    ggggtgaagt cgtaacaagg taaa
                                                                       1524
30
    <210> 28
     <211> 1555
     <212> DNA
     <213> Staphylococcus aureus
35
    <400> 28
    ttttatggag agtttgatcc tggctcagga tgaacgctgg cggcgtgcct aatacatgca 60
    agtegagega acggacgaga agettgette tetgatgtta geggeggaeg ggtgagtaac 120
    acqtqqataa cctacctata agactqqqat aacttcqqqa aaccqqaqct aataccqqat 180
    aatattttga accgcatggt tcaaaagtga aagacggtct tgctgtcact tatagatgga 240
    teegegetge attagetagt tggtaaggta acggettace aaggeaacga tacgtageeg 300
    acctgagagg gtgatcggcc acactggaac tgagacacgg tccagactcc tacgggaggc 360
    agcagtaggg aatcttccgc aatgggcgaa agcctgacgg agcaacgccg cgtgagtgat 420
    gaaggtette ggategtaaa actetgttat tagggaagaa catatgtgta aqtaactqtg 480
    cacatettga eggtacetaa teagaaagee aeggetaaet aegtgeeage ageegeggta 540
    atacqtaqqt qqcaaqcqtt atccqqaatt attqqqcqta aaqcqcqcqt aqqcqqtttt 600
    ttaaqtctqa tqtqaaaqcc cacqqctcaa ccqtqqaqqq tcattqqaaa ctqqaaaact 660
    tqaqtqcaqa aqaqqaaaqt qqaattccat qtqtaqcqqt qaaatqcqca qaqatatqqa 720
    ggaacaccag tggcgaaggc gactttctgg tctgtaactg acgctgatgt gcgaaagcgt 780
    ggggatcaaa caggattaga taccctggta gtccacgccg taaacgatga gtgctaagtg 840
50
    ttagggggtt tccgccctt agtgctgcag ctaacgcatt aagcactccg cctggggagt 900
    acgaccgcaa ggttgaaact caaaggaatt gacggggacc cgcacaagcg gtggagcatg 960
    tggtttaatt cgaagcaacg cgaagaacct taccaaatct tgacatcctt tgacaactct 1020
    agagatagag cetteccett egggggacaa agtgacaggt ggtgcatggt tgtegteage 1080
    togtgtcgtg agatgttggg ttaagtcccg caacgagcgc aaccettaag cttagttgcc 1140
55
    atcattaagt tgggcactct aagttgactg ccggtgacaa accggaggaa ggtggggatg 1200
    acqtcaaatc atcatqcccc ttatqatttq qqctacacac qtqctacaat qqacaataca 1260
```

WO 03/054162 PCT/US02/41014 10/52

```
aagggcagcg aaaccgcgag gtcaagcaaa tcccataaag ttgttctcag ttcgqattqt 1320
     agtotgoaac togactacat gaagotggaa togotagtaa togtagatoa qoatgotacq 1380
     gtgaatacgt tcccgggtat tgtacacacc gcccgtcaca ccacgagagt ttgtaacacc 1440
     cgaaqccggt ggagtaacct tttaggagct agccgtcgaa ggtgggacaa atgattgggg 1500
     tgaagtcgta acaaggtagc cgtatcggaa ggtgcggctg gatcacctcc tttct
     <210> 29
    <211> 1551
10
   <212> DNA
     <213> Streptococcus mutans
     <400> 29
    agagtttgat cctggctcag gacgaacgct ggcggcgtgc ctaatacatg caagtgggac 60
    gcaaggaaac acactgtgct tgcacaccgt gttttcttga gtcgcgaacg ggtgagtaac 120
    gcgtaggtaa cctgcctatt agcgggggat aactattgga aacgatagct aataccgcat 180
     aatattaatt attgcatgat aattgattga aagatgcaag cgcatcacta gtagatggac 240
     ctgcgttgta ttagctagtt ggtaaggtaa gagcttacca aggcgacgat acatagccga 300
     cctgagaggg tgatcggcca cactgggact gagacacggc ccagactcct acgggaggca 360
20
   gcagtaggga atcttcggca atggacgaaa gtctgaccga gcaacgccgc gtgagtgaag 420
    aaggttttcg gatcgtaaag ctctgttgta agtcaagaac gtgtgtgaga gtggaaagtt 480
    cacacagtga cggtagctta ccagaaaggg acggctaact acgtgccagc agccgcggta 540
    atacgtaggt cccgagcgtt gtccggattt attgggcgta aagggagcgc aggcggtcag 600
    gaaagtctgg agtaaaaggc tatggctcaa ccatagtgtg ctctggaaac tgtctgactt 660
    gagtgcagaa ggggagagtg gaattccatg tgtagcggtg aaatgcgtag atatatggag 720
    gaacaccagt ggcgaaagcg gctctctggt ctgtcactga cgctgaggct cgaaagcgtg 780
    ggtagcgaac aggattagat accctggtag tccacgccgt aaacgatgag tgctaggtgt 840
     taggcccttt ccggggctta gtgccggagc taacgcaata agcactccgc ctggggagta 900
    cgaccgcaag gttgaaactc aaaggaattg acgggggccc gcacaagcgg tggagcatgt 960
30
    ggtttaattc gaagcaacgc gaagaacctt accaggtctt gacatcccga tgctattctt 1020
    agagatagga agttacttcg gtacatcgga gacaggtggt gcatggttgt cgtcagctcg 1080
    tgtcgtgaga tgttgggtta agtcccgcaa cgagcgcaac ccttattgtt agttgccatc 1140
    attaagttgg gcactctagc gagactgccg gtaataaacc ggaggaaggt ggggatgacg 1200
    tcaaatcatc atgcccctta tgacctgggc tacacacgtg ctacaatggt cggtacaacg 1260
    agttgcgagc cggtgacggc aagctaatct ctgaaagccg atctcagttc ggattggagg 1320
    ctgcaactcg cctccatgaa gtcggaatcg ctagtaatcg cggatcagca cgccgcggtg 1380
    aatacgttcc cgggccttgt acacaccgcc cgtcacacca cgagagtttg taacacccga 1440
    agtoggtgag gtaacctttt aagggccaag cogcctaagg tgggatggat gattggggtg 1500
    aagtcgtaac aaggtagccg tatcggaagg tgcggctgga tcacctcctt t
40
    <210> 30
    <211> 1515
    <212> DNA
45
    <213> Streptococcus pneumoniae
    <400> 30
    atttgatect ggeteaggae gaacgetgge ggegtgeeta atacatgeaa gtagaacget 60
    gaaggaggag cttgcttctc tggatgagtt gcgaacgggt gagtaacgcg taggtaacct 120
    gcctggtagc gggggataac tattggaaac gatagctaat accgcataag agtggatgtt 180
    gcatgacatt tgcttaaaag gtgcacttgc atcactacca gatggacctq cgttgtatta 240
    gctagttggt ggggtaacgg ctcaccaagg cgacgataca tagccgacct gagagggtga 300
    teggecacae tgggaetgag acaegkeeca gaeteetaeg ggaggeagea gtagggaate 360
    ttcggcaatg gacggaagtc tgaccgagca acgccgcgtg agtgaagaag gttttcggat 420
55
    cqtaaaqctc tqttqtaaqa qaaqaacqaq tqtqaqaqtq qaaaqttcac actqtqacqq 480
    tatettacca gaaaggacg getaactacg tgccagcage cgcggtaata cgtaggteec 540
```

55 <220>

```
gagcgttgtc cggatttatt gggcgtaaag cgagcgcagg cggttagata agtctgaagt 600
     taaaggotgt ggottaacca tagtaggott tggaaactgt ttaacttgag tgcaagaggg 660
     qaqaqtqqaa ttccatqtqt aqcqqtqaaa tqcqtagata tatggaggaa caccqqtggc 720
     qaaaqcqqct ctctqqcttq taactqacqc tqaqqctcqa aaqcqtgqgq agcaaacaqq 780
     attagatace etggtagtee accetgtaaa egatgagtge taggtgttag accettteeg 840
     qqqtttaqtq ccqtaqctaa cqcattaagc actccqcctq qqqattcqa ccqcaaqqtt 900
     gaaactcaaa ggaattgacg ggggcccgca caagcggtgg agcatgtggt ttaattcqaa 960
    gcaacgcgaa gaaccttacc aggtcttgac atccctctga ccgctctaga gatagagttt 1020
    teetteggga cagaggtgac aggtggtgca tggttgtegt cagetegtgt cgtgagatgt 1080
10
    tgggttaagt cccgcaacga gcgcaaccc tattgttagt tgccatcatt cagttgggca 1140
    ctctaqcqaq actqccqqta ataaaccqqa qqaaqqtqqq qatqacqtca aatcatcatq 1200
     ccccttatga cctqqqctac acacqtqcta caatqqctqq tacaacqaqt cqcaaqccqq 1260
     tgacggcaag ctaatctctt aaagccagtc tcagttcgga ttgtaggctg caactcgcct 1320
    acatgaagtc ggaatcgcta gtaatcgcgg atcagcacgc cgcggtgaat acgttcccgg 1380
    gccttgtaca caccgcccgt cacaccacga gagtttgtaa cacccgaagt cggtgaggta 1440
    accgtaagga gccagccgcc taaggtggga tagatgattg gggtgaagtc gtaacaaggt 1500
     cagccgtttg ggaga
20
    <210> 31
    <211> 1335
     <212> DNA
    <213> Streptococcus pyogenes
25 <400> 31
    gaacgggtga gtaacgcgta ggtaacctac ctcatagcgg gggataacta ttggaaacga 60
    tagctaatac cgcataagag agactaacgc atgttagtaa tttaaaaggg gcaattgctc 120
    cactatgaga tggacctgcg ttgtattagc tagttggtga ggtaaaggct caccaaggcg 180
    acgatacata geogaeetga gagggtgate ggecacactg ggaetgagae acggeceaga 240
    ctcctacqqq aqqcaqcaqt aqqqaatctt cqqcaatqqq qqcaaccctg accqaqcaac 300
    gccgcgtgag tgaagaaggt tttcggatcg taaagctctg ttgttagaga agaatgatgg 360
    tqqqaqtqqa aaatccacca agtgacggta actaaccaga aaqqqacggc taactacgtg 420
    ccagcagecg eggtaatacg taggteecga gegttgteeg gatttattgg gegtaaageg 480
    agggcaggcg gttttttaag tctgaagtta aaggcattgg ctcaaccaat gtacgctttg 540
35
    gaaactggag aacttgagtg cagaagggga gagtggaatt ccatgtgtag cggtgaaatg 600
    cgtagatata tggaggaaca ccggtggcga aagcggctct ctggtctgta actgacgctg 660
    aggetegaaa gegtggggag caaacaggat tagataccet ggtagtecac geegtaaacg 720
    atgagtgcta ggtgttaggc cctttccggg gcttagtgcc ggagctaacg cattaagcac 780
    tccgcctggg gagtacgacc gcaaggttga aactcaaagg aattgacggg ggcccgcaca 840
40
    ageggtggag catgtggttt aattegaage aacgegaaga acettaceag gtettgacat 900
    cccgatgccc gctctagaga tagagtttta cttcggtaca tcggtgacag gtggtgcatg 960
    gttqtcqtca gctcqtqtcq tgagatqttq ggttaagtcc cgcaacqagc gcaaccccta 1020
    ttgttagttg ccatcattaa gttgggcact ctagcgagac tgccggtaat aaaccggagg 1080
    aaggtgggga tgacgtcaaa tcatcatgcc ccttatgacc tgggctacac acgtgctaca 1140
45
    atogttogta caacqagtcg caagccggtg acggcaagct aatctcttaa agccaatctc 1200
    agtteggatt gtaggetgea actegectae atgaagtegg aategetagt aategeggat 1260
    cagcacgccg cggtgaatac gttcccggc cttgtacaca ccgcccgtca caccacgaga 1320
    gtttgtaaca cccga
50
    <210> 32
    <211> 1465
    <212> DNA
    <213> Mycobacterium avium
```

WO 03/054162 PCT/US02/41014 12/52

```
<221> modified base
    <222> (298)..(881)
    <223> N = A, C, G or T/U
    <400> 32
    ggcggcgtgc ttaacacatg caagtcgaac ggaaaggcct cttcggaggt actcgagtgg 60
    cgaacgggtg agtaacacgt gggcaatcta ccctgcactt cgggataagc ctgggaaact 120
    qqqtctaata ccggatagga cctcaagacg catgtcttct ggtggaaagc ttttgcggtg 180
    tgggatgggc ccgcggccta tcagcttgtt ggtggggtga cggcctacca aggcgacgac 240
    qqqtaqccqq cctgaqaqqq tqtccqqcca cactqqqact gagatacqqc ccagactnct 300
    acgggaggca gcagtgggga atattgcaca atgggcgcaa gcctgatgca gcgacgccgc 360
    gtgggggatg acggccttcg ggttgtaaac ctctttcacc atcgacgaag gtccgggttt 420
    tctcggattg acggtaggtg gagaagaagc accggccaac tacgtgccag cagccgcqct 480
    aatacgtagg gtgcgagcgt tgtccggaat tactgggcgt aaagagctcg taggtggttt 540
15
    qtcqcqttqt tcqtgaaatc tcacqqctta actgtgagcg tgcgngcgat acgggcagac 600
    tagagtactg caggggagac tggaattcct ggtgtagcgg tggaatgcgc agatatcagg 660
    aggaacaccg gtggcgaagg cgggtctctg ggcagtaact gacgctgagg agcgaaagcg 720
    tggggagega acaggattag ataccetggt agtecacgne gtaaacggtg ggtactaggt 780
    gtgggtttcc ttccttggga tccgtgccgt agctaacgca ttaagtaccc cgcctgggga 840
20
    qtacqqncqc aaqqctaaaa ctcaaaqqaa ttqacqqqqq nccqcacaaq cggcggagca 900
    tgtggattaa ttcgatgcaa cgcgaagaac cttacctggg tttgacatgc acaggacgcg 960
    tctaqaqata qqcqttccct tqtqqcctqt qtqcaqqtqq tqcatgqctg tcqtcagctc 1020
    gtgtcgtgag atgttgggtt aagtcccgca acgagcgcaa cccttgtctc atgttgccag 1080
    cqqqtaatqc cqqqqactcq tgagagactg ccggggtcaa ctcggaggaa ggtggggatg 1140
25
    acgtcaagtc atcatgcccc ttatgtccag ggcttcacac atgctacaat ggccggtaca 1200
    aagggctgcg atgccgtaag gttaagcgaa tccttttaaa gccggtctca gttcggattg 1260
    gggtctgcaa ctcgacccca tgaagtcgga gtcgctagta atcgcagatc agcaacgctg 1320
    cogtquatac gttcccqqqc cttqtacaca ccqcccqtca cqtcatquaa gtcgqtaaca 1380
    cccgaagcca gtggcctaac ccttttggga gggagctgtc gaaggtggga tcggcgattg 1440
30 ggacgaagtc gtaacaaggt agccg
    <210> 33
    <211> 1536
35
    <212> DNA
    <213> Mycobacterium tuberculosis
    <400> 33
    tttgtttgga gagtttgatc ctggctcagg acgaacgctg gcggcgtgct taacacatgc 60
40
    aagtcgaacg gaaaggtctc ttcggagata ctcgagtggc gaacgggtga gtaacacgtg 120
    ggtgatctgc cctgcacttc gggataagcc tgggaaactg ggtctaatac cggataggac 180
    cacgggatgc atgtcttgtg gtggaaagcg ctttagcggt gtgggatgag cccgcggcct 240
    atcagcttgt tggtggggtg acggcctacc aaggcgacga cgggtagccg gcctgagagg 300
    qtqtccqqcc acactqqqac tqaqatacqq cccaqactcc tacqqqaggc agcagtqggg 360
45
    aatattgcac aatgggcgca agcctgatgc agcgacgccg cgtgggggat gacggccttc 420
    gggttgtaaa cctctttcac catcgacgaa ggtccgggtt ctctcggatt gacggtaggt 480
    qqaqaaqaaq caccqqccaa ctacqtqcca qcaqccqcqq taatacgtag ggtgcgagcg 540
    ttgtccggaa ttactgggcg taaagagctc gtaggtggtt tgtcgcgttg ttcgtgaaat 600
    ctcacggctt aactgtgagc gtgcgggcga tacgggcaga ctagagtact gcaggggaga 660
    ctggaattcc tggtgtagcg gtggaatgcg cagatatcag gaggaacacc ggtggcgaag 720
50
    gcgggtctct gggcagtaac tgacgctgag gagcgaaagc gtggggagcg aacaggatta 780
    qataccetqq taqtccacqc cgtaaacqgt gggtactagg tgtgggtttc cttccttggg 840
    atccgtgccg tagctaacgc attaagtacc ccgcctgggg agtacggccg caaggctaaa 900
    actcaaagga attgacgggg gcccgcacaa gcggcggagc atgtggatta attcgatgca 960
55
    acqcgaagaa ccttacctgg gtttgacatg cacaggacgc gtctagagat aggcgttccc 1020
     ttgtggcctg tgtgcaggtg gtgcatggct gtcgtcagct cgtgtcgtga gatgttgggt 1080
```

```
taagtcccgc aacgagcgca accettgtct catgttgcca gcacgtaatg gtggggactc 1140
    qtgaqaqact gccggggtca actcggagga aggtggggat gacgtcaagt catcatgccc 1200
    cttatgtcca gggcttcaca catgctacaa tggccggtac aaagggctgc gatgccgcga 1260
    ggttaagega atcettaaaa geeggtetea gtteggateg gggtetgeaa etegaceeeg 1320
    tgaagtcgga gtcgctagta atcgcagatc agcaacgctg cggtgaatac gttcccgggc 1380
    cttqtacaca ccqcccqtca cqtcatqaaa qtcqqtaaca cccqaagcca gtggcctaac 1440
    cctcqqqaqq qaqctqtcqa aqqtqqqatc qqcqattqqq acqaaqtcqt aacaaggtag 1500
    ccqtaccqqa agqtqcggct gqatcacctc ctttct
10
    <210> 34
    <211> 1536
    <212> DNA
     <213> Escherichia coli
15
    <400> 34
    tttgtttgga gagtttgatc ctggctcagg acgaacgctg gcggcgtgct taacacatgc 60
    aaqtcgaacq qaaaqgtctc ttcggagata ctcgagtggc gaacgggtga gtaacacgtg 120
    ggtgatctgc cctgcacttc gggataagcc tgggaaactg ggtctaatac cggataggac 180
20
    cacgggatgc atgictigtg giggaaagcg ctttagcggt gigggatgag cccgcggcct 240
    atcagettqt tqqtqqqtq acqqcctacc aaqqcqacqa cqqqtaqccq gcctqagagg 300
    gtgtccggcc acactgggac tgagatacgg cccagactcc tacgggaggc agcagtgggg 360
    aatattgcac aatgggcgca agcctgatgc agcgacgccg cgtgggggat gacggccttc 420
    gggttgtaaa cctctttcac catcgacgaa ggtccgggtt ctctcggatt gacggtaggt 480
25
    qqaqaaqaaq caccqqccaa ctacqtqcca gcaqccqcqg taatacqtaq qqtqcqaqcq 540
    ttgtccggaa ttactgggcg taaagagctc gtaggtggtt tgtcgcgttg ttcgtgaaat 600
    ctcacqqctt aactgtgagc gtgcgggcga tacgggcaga ctagagtact gcaggggaga 660
    ctggaattcc tggtgtagcg gtggaatgcg cagatatcag gaggaacacc ggtggcgaag 720
    gcgggtctct gggcagtaac tgacgctgag gagcgaaagc gtggggagcg aacaggatta 780
    gataccetgg tagtccacge cgtaaacggt gggtactagg tgtgggttte cttccttggg 840
    atcoptgccq tagctaacgc attaagtacc ccqcctgggg agtacggccg caaggctaaa 900
    actcaaagga attgacgggg gcccgcacaa gcggcggagc atgtggatta attcgatgca 960
    acqcqaaqaa ccttacctgg gtttgacatg cacaggacgc gtctagagat aggcgttccc 1020
    ttgtqqcctq tqtqcaqqtg gtgcatggct gtcgtcagct cgtgtcgtga gatgttgggt 1080
35 taagtcccgc aacgagcgca accettgtet catgttgcca gcacgtaatg gtggggactc 1140
    gtgagagact gccggggtca actcggagga aggtggggat gacgtcaagt catcatgccc 1200
    cttatgtcca gggcttcaca catgctacaa tggccggtac aaagggctgc gatgccgcga 1260
    ggttaagcga atccttaaaa gccggtctca gttcggatcg gggtctgcaa ctcgaccccg 1320
    tgaagtcgga gtcgctagta atcgcagatc agcaacgctg cggtgaatac gttcccgggc 1380
    cttgtacaca ccgcccgtca cgtcatgaaa gtcggtaaca cccgaagcca gtggcctaac 1440
    cctcqqqagg gagctgtcga aggtgggatc ggcgattggg acgaagtcgt aacaaggtag 1500
    ccgtaccgga aggtgcggct ggatcacctc ctttct
45
    <210> 35
     <211> 1534
     <212> DNA
    <213> Klebsiella pneumoniae
    <220>
    <221> modified base
    <222> (11)..(12)
    <223> N = A. C. G or T/U
55
```

agagtttgat nntggctcag attgaacgct ggcggcaggc ctaacacatg caagtcgagc 60

WO 03/054162 PCT/US02/41014

```
ggtagcacag agagcttgct ctcgggtgac gagcggcgga cgggtgagta atgtctggga 120
     aactgcctga tggagggga taactactgg aaacggtagc taataccgca taacgtcgca 180
     agaccaaagt gggggacctt cgggcctcat gccatcagat gtgcccagat gggattagct 240
     agtaggtggg gtaacggctc acctaggcga cgatccctag ctggtctgag aggatgacca 300
     qccacactqq aactqaqaca cqqtccaqac tcctacqqqa qqcaqcaqtq qqqaatattq 360
     cacaatqqqc qcaaqcctqa tqcaqccatq ccqcqtqtqt gaaqaaqqcc ttcqqqttqt 420
     aaagcacttt cagcggggag gaaggcgatg aggttaataa cctcatcgat tgacgttacc 480
     ctqcaqaaqa aqcaccqqct aactccqtgc cagcagccgc ggtaatacgg agggtgcaag 540
     cgttaatcgg aattactggg cgtaaagcgc acgcaggcgg tctgtcaagt cggatgtgaa 600
10 atccccgggc tcaacctggg aactgcattc gaaactggca ggctagagtc ttgtagaggg 660
     gggtagaatt ccaggtgtag cggtgaaatg cgtagagatc tggaggaata ccggtggcga 720
     aggeggeece etggacaaag actgaegete aggtgegaaa gegtggggag caaacaggat 780
     tagataccct ggtagtccac gccgtaaacg atgtcgattt ggaggttgtg cccttgaggc 840
     gtggcttccg gagctaacgc gttaaatcga ccgcctgggg agtacggccg caaggttaaa 900
15
     actcaaatga attgacgggg gcccgcacaa gcggtggagc atgtggttta attcgatgca 960 acgcgaagaa ccttacctgg tcttgacatc cacagaactt tccagagatg gattggtgcc 1020
     ttcgggaact gtgagacagg tgctgcatgg ctgtcgtcag ctcgtgttgt gaaatgttgg 1080
     gttaagtccc gcaacgagcg caacccttat cctttgttgc cagcggttag gccgggaact 1140
     caaaqqaqac tqccaqtqat aaactqqaqq aaqqtqqqqa tqacqtcaaq tcatcatqqc 1200
     ccttacqacc aqqqctacac acqtqctaca atqqcatata caaaqaqaaq cqacctcqcq 1260
     agagcaagcg gacctcataa agtatgtcgt agtccggatt ggagtctgca actcgactcc 1320
     atqaaqtcqq aatcqctaqt aatcqtaqat caqaatqcta cggtqaatac gttcccgggc 1380
     cttqtacaca ccqcccgtca caccatggga gtgggttgca aaagaagtag gtagcttaac 1440
     cttcqqqaqq qcqcttacca ctttgtgatt catgactqqq gtgaagtcgt aacaaggtaa 1500
25
     ccgtagggga acctgcggtt ggatcacctc cttt
     <210> 36
     <211> 1485
30
     <212> DNA
     <213> ACTINOBACCILUS ACTIN
     <221> modified base
35
     <222> (208)..(1476)
     <223> N = A, C, G or T/U
     <400> 36
     attgaagagt ttgatcatgg ctcagattga acgctggcgg caggcttaac acatgcaagt 60
     cggacggtag caggagaaag cttgctttct tgctgacgag tggcggacgg gtgagtaatg 120
     cttgggaatc tgtcttatgg agggggataa cgacgggaaa ctgtcgctaa taccgcgtag 180
     aqtcqqqaqa cqaaaqtqcq qqactttntq qccqcatqcc atqaqatqaq cccaaqtqtq 240
     attaggtagt tggtggggta aaggcctacc aagccgacga tcgctagctg gtctgagagg 300
     atggccagec acaccgggac tgagacacgg cccngactec tacgggaggc agcagtgggg 360
    aatattqcqc aatqqqqqca accetgacqc agccatqccq cqtqaatqaa qaagqccttc 420
     gggttqtaaa qttctttcqq tattqaqqaa ggttggtgt ttaatagcat gccaaattga 480
     cqttaaatac agaaqaaqca ccqgctaact ccgtgccaqc agccgcggta atacgggggg 540
     tgcgaqcgtt aatcggaata actgggcgta aagggcacgt aggcggacct ttaaqtgaqg 600
     tgtgaaatcc ccgggcttaa cctgggnatt gcatttcata ctgggggtct ggagtacttt 660
    ngggagggnt agaattccac gtgtagcggt gaaatgcgta gagatgtgga ggaataccga 720
     aggcgaaggc agccccttgg ggatgtactg acgctgatgt gcgaaagcgt ggggagcaaa 780
     caggattaga taccctogta qtccacqctq taaacqqtqt cqatttqqqq attqqqqttt 840
     agccctggtg cccgaagcta acgtgataaa tcgaccgcct ggggagtacg gccgcaaggt 900
     taaaactcaa atgaattgac gggggcccgc acaagcggtg gagcatgtgg tttaattcga 960
    tqcaacqcqa agaaccttac ctactcttga catccqaaqa agaactcaga gatgggtttg 1020
     tqccttaggg agctttgaga caggtgctgc atggcngtcg tcagctcqtg ttgtgaaatg 1080
```

```
ttqqqttaag tcccgcaacg agcgcaaccc ttatcctttg tggccagcga cgtggtcggg 1140
    aactcaaagg agactgccgg tgataaaccg gaggaaggtg gggatgacgt caagtcatca 1200
    tgqcccttac gagtagggct acacacgtgc tacaatggcg tatacagagg gtaaccaacc 1260
    aggatggg agtgaatete agaaagtgeg tetaagtteg gattggagte tgeaactega 1320
    ctccatgaag teggaatege tagtaatege gaateagaat gttgeggtga ataegtteee 1380
    qqqccttqta cacaccqccc gtcacaccat gggagtgggt tgtaccagaa qtqqatagct 1440
    gaaccgagag ggtggcgttt accacggtat gattcangac tgggg
10 <210> 37
    <211> 1487
    <212> DNA
    <213> Haemophilus influenzae
15 <220>
    <221> modified_base
    <222> (1) . . (1387)
    <223> N = A, C, G or T/U
20 <400> 37
    naattgaaga gtttgatcat ggctcagatt gaacgctggc ggcaggctta acacatgcaa 60
    gtcgaacggt agcaggagaa agcttgcttt cttgctgacg agtggcggac gggtgagtaa 120
    tgcttgggaa tctggcttat ggagggggat aacgacggga aactgtcgct aataccgcgt 180
    attatoggaa gatgaaagtg ogggactgag aggcogcatg ccataggatg agcccaagtg 240
    ggattaggta gttggtgggg taaatgccta ccaagcctgc gatctctagc tggtctgaga 300
    ggatgaccag ccacactgga actgagacac ggtccagact cctacgggag gcagcagtgg 360
    ggaatattgc gcnatggggg gaaccctgac gcagccatgc cgcgtgaatg aagaaggcct 420
    tegggttgta aagttettte ggtattgagg aaggttgatg tgttaatage acateaaatt 480
    qacqttaaat acagaagaag caccggctaa ctccgtgcca gcagccgcgg taatacggag 540
30 notgcgagcg ttaatcggaa taactgggcg taaagggcac gcaggcggtt atttaagtga 600
    ggtgtgaaag ccccgggctt aacctgggna ttgcatttca gactgggtaa ctagagtact 660
    ttagggaggg gtagaattcc acgtgtagcg gtgaaatgcg tagagatgtg gaggaatacc 720
    gaaggegaag geageeeett gggaatgtae tgaegeteat gtgegaaage gtggggagea 780
    aacaggatta gataccetgg tagtecacge tgtaaacget gtegatttgg gggttggggt 840
35 ttaactotgg caccogtago taacgtgata aatcgaccgo otggggagta oggoogcaag 900
    qttaaaactc aaatgaattg acgggggcon gcacaagegg tggagcatgt ggtttaattc 960
    gatgcaacgc gaagaacctt acctactctt gacatcctaa gaagagctca gagatgagct 1020
    tgtgccttcg ggaacttaga gacaggtgct gcatggctgt cgtcagctcg tgttgtgaaa 1080
    tgttgggtta agtcccgcaa cgagcgcaac ccttatcctt tgttgccagc gacttggtcg 1140
    ggaactcaaa ggagactgcc agtgataaac tggaggaagg tngggatgac gtcaagtcat 1200
    catggccctt acgagtaggg ctacacacgt gctacaatgg cgtatacaga gggaagcgaa 1260
    qctqcqaqqt qqaqcqaatc tcataaaqta cqtctaaqtc cqqattqqaq tctqcaactc 1320
    gactocatga agtoggaato gotagtaato gogaatoaga atgtogoggt gaatacgtto 1380
    coqqqcnttq tacacaccgc ccgtcacacc atgggagtgg gttgtaccag aagtagatag 1440
45 cttaaccttt tggagggcgt ttaccacggt atgattcatg actgggg
    <210> 38
    <211> 1532
    <212> DNA
    <213> Bordetella bronchiseptica
    <400> 38
    tgaactgaag agtttgatcc tgqctcaqat tgaacgctgg cgggatqctt tacacatgca 60
55
    agtcqqacqq caqcacqqqc ttcqqcctqq tqgcgagtgg cgaacgggtg agtaatgtat 120
    cqqaacqtqc ccaqtaqcgq qqqataacta cqcgaaaqcq tgqctaatac cqcatacqcc 180
```

PCT/US02/41014

```
ctacggggga aagcggggga ccttcgggcc tcqcactatt ggagcggccg atatcggatt 240
     agctagttgg tggggtaacg gcctaccaag gcgacgatcc gtagctggtt tgagaggacq 300
     accagecaca etgggaetga gacaeggeee agaeteetae gggaggeage agtggggaat 360
     tttggacaat gggggCaacc ctgatccagc catcccgcgt gtgcgatgaa ggccttcggg 420
     ttqtaaaqca cttttqqcaq qaaaqaaacq qcacqqqcta atatcctqtq caactqacqq 480
     tacctgcaga ataagcaccg gctaactacg tgccagcagc cgcggtaata cgtagggtgc 540
     aagcgttaat cggaattact gggcgtaaag cgtgcgcagg cggttcggaa agaaagatgt 600
    gaaatcccag ggcttaacct tggaactgca tttttaacta ccgggctaga gtgtgtcaga 660
    qqqaqqtgqa attccgcgtg tagcagtgaa atqcqtaqat atqcqqaqqa acaccqatqq 720
    cgaaggcagc ctcctgggat aacactgacg ctcatqcacq aaagcqtqqq qaqcaaacaq 780
    gattagatac cotggtagtc cacgccctaa acgatgtcaa ctagctgttq qqqccttcqq 840
    gccttggtag cgcagctaac gcgtgaagtt gaccgcctgg ggagtacggt cqcaagatta 900
    aaactcaaag gaattgacgg ggacccgcac aagcggtgga tgatgtggat taattcgatg 960
    caacgcgaaa aaccttacct acccttgaca tgtctggaat cccgaagaga tttgggagtg 1020
    ctcgcaagag aaccggaaca caggtgctgc atggctgtcg tcagctcgtg tcgtgagatg 1080
    ttgggttaag tcccgcaacg agcgcaaccc ttgtcattag ttgctacgaa agggcactct 1140
    aatgagactg ccggtgacaa accggaggaa ggtggggatg acgtcaagtc ctcatggccc 1200
    ttatgggtag ggcttcacac gtcatacaat ggtcgggaca gagggtcgcc aacccgcgag 1260
    ggggagccaa tcccagaaac ccgatcgtag tccggatcgc agtctgcaac tcgactqcgt 1320
    gaagtoggaa togotagtaa togoggatoa goatgtogog gtgaataogt tocogggtot 1380
    tgtacacacc gcccgtcaca ccatgggagt gggttttacc agaagtagtt agcctaaccg 1440
    caagggggc gattaccacg gtaggattca tgactggggt gaagtcgtaa caaggtagcc 1500
    gtatcggaag gtgcggctgg atcacctcct tt
25
    <210> 39
    <211> 1485
    <212> DNA
    <213> Bordetella parapertussis
30
    <400> 39
    attgaacgct ggcgggatgc tttacacatg caagtcggac ggcagcacgg gcttcggcct 60
    ggtggcgagt ggcgaacggg tgagtaatgt atcggaacgt gcccagtagc gggggataac 120
    tacgcgaaag cgtggctaat accgcatacg ccctacgggg gaaagcgggg gactttcggg 180
35
    cctcgcacta ttggagcggc cgatatcgga ttagctagtt ggtggggtaa cggcctacca 240
    aggegaegat cegtagetgg tttgagagga egaceageea caetgggaet gagaeaegge 300
    ccagactect acgggaggca gcagtgggga attttggaca atgggggcaa ccctgatcca 360
    gccatcccgc gtgtgcgatg aaggccttcg ggttgtaaag cacttttggc aggaaagaaa 420
    cggcacgggc taatatcctg tgcaactgac ggtacctgca gaataagcac cggctaacta 480
40
    cgtgccagca gccgcggtaa tacgtagggt gcaagcgtta atcggaatta ctgggcgtaa 540
    agcgtgcgca ggcggttcgg aaagaaagat gtgaaatccc agggcttaac cttggaactg 600
    catttttaac taccgggcta gagtgtgtca gagggaggtg gaattccgcg tgtagcagtg 660
    aaatgcgtag atatgcggag gaacaccgat ggcgaaggca gcctcctggg ataacactga 720
    cgctcatgca cgaaagcgtg gggagcaaac aggattagat accctggtag tccacgccct 780
    aaacgatgtc aactagctgt tggggccttc gggccttggt agcgcagcta acgcgtgaag 840
    ttgaccgcct ggggagtacg gtcgcaaqat taaaactcaa aggaattgac ggggacccgc 900
    acaagcggtg gatgatgtgg attaattcga tgcaacgcga aaaaccttac ctacccttga 960
    catgtctgga atcccgaaga gatttgggag tgctcgcaag agaaccggaa cacaggtgct 1020
    gcatggctgt cgtcagctcg tgtcgtgaga tgttgggtta agtcccgcaa cgagcgcaac 1080
    ccttgtcatt agttgctacg aaagggcact ctaatgagac tgccggttac aaaccggagg 1140
    aaggtgggga tgacgtcaag tcctcatggc ccttatgggt agggcttcac acgtcataca 1200
    atggteggga cagagggteg ccaaccegeg agggggagec aatcccagaa accegategt 1260
    agteeggate geagtetgea actegactge gtgaagtegg aategetagt aategeggat 1320
    cagcatgtcg cggtgaatac gttcccgggt cttgtacaca ccgcccgtca caccatggga 1380
    gtgggtttta ccagaagtag ttagcctaac cgcaaggggg gggcgattac cacggtagga 1440
    ttcatgactg gggtgaagtc gtaacaaggt agccgtatcg gaagg
```

```
<210 > 40
     <211> 1464
     <212 > DNA
     <213> Bordetella pertussis
    <221> modified base
10
    <222> (87) .. (1391)
     <223> N = A. C. G or T/U
     <400> 40
    aactgaagag tttgatcctg gctcagattg aacgctggcg ggatgcttta cacatgcaag 60
15
    teggacqqca qcacqqqctt cqqcctnqtq qcqaqtqqcq aacqqqtqaq taatqtatcq 120
    gaacgtgccc agtagcgggg gataactacg cgaaagcgta gctaataccg catacgccct 180
    acgggggaaa gcgggggacc ttcgggcctc gcactattgg agcggccgat atcggattag 240
    ctngttggtg gggtaacggc ctaccaaggc gacgatccgt agctggtttg agaggacgac 300
    cagccacact gggactgaga cacggcccag nctcctacgg gaggcagcag tggggaattt 360
20
   tggacaatgg gggcaaccct gatccagcca tcccgcgtgt gcgatgaagg ccttcgggtt 420
    qtaaaqcact tttqqcaqqa aaqaaacqqc acqqqctaat atcctqtqca actqacqqta 480
    cctqcaqaat aaqcaccqqc taactacqtq ccaqcaqccq cqqtaatacq taqqqtqcaa 540
    qcqttaatcq qaattactgg gcgtaaaqcq tqcqcaqqcq qttcqqaaaq aaaqatqtqa 600
    aatcccaggg cttaaccttg gaactgcatt tttaactacc gggctagagt gtgtcagagg 660
    qaqqtqqaat teegegtqta geaqtqaaat qeqtaqatat qeqqaqqaac accqatqqeq 720
    aaggcagcct cctgggataa cactgacgct catgcacgaa agtgtgggga gcaaacagga 780
    ttagataccc tggtagtcca cgccctaaac gatgtcaact agctgttggg gccttcgggc 840
    cttggtagcg cagctaacgc gtgaagttga ccgcctgggg agtacggtcg caagattaaa 900
    actcaaagga attgacgggg acccgcacaa gcggtggatg atgtggatta attcgatgca 960
   acgcgaaaaa ccttacctac ccttgacatg tctggaatcc cgaagagatt tgggagtgct 1020
    cgcaagagaa ccggaacaca ggtgctgcat ggctgtcgtc agctcgtgtc gtgagatgtt 1080
    gggttaagtc ccgcaacgag cgcaaccctt gtcattagtt gctacgaaag ggcactctaa 1140
    tgagactgcc ggtgacaaac cggaggaagg tggggatgac gtgaagtcct catggccctt 1200
    atgggtaggg cttcacacgt catacaatgg tcgggacaqa gggttgncaa cccgcgaggg 1260
35
    ggagccaatc ccagaaaccc ggtcgtngtc cggatcgcag tctgcaactc gactgcgtga 1320
    agtoggaato gotagtaato goggatoago atgtogoggt gaatacgtto cogggtottg 1380
    tacacaccgc negteacacc atgggagtgg gttttaccag aagtagttag cctaaccgca 1440
    aggggggga ttaccacggt agga
40
    <210> 41
    <211> 1535
    <212> DNA
    <213> Burkholderia cepacia
45
    <400> 41
    taaactgaag agtttgatcc tggctcagat tgaacgctgg cggcatgctt aacacatgca 60
    agtegaacgg cagcacgggt gcttgcacct ggtggcgagt ggcgaacggg tgagtaatac 120
    ateggaacat gteetgtagt gggggatage eeggegaaag eeggattaat acegcataeg 180
50
    atctacggat gaaagcgggg gaccttcggg cctcgcgcta tagggttggc gatggctgat 240
    tagctagttg gtggggtaaa ggcctaccaa ggcgacgatc agtagctggt ctgagaggac 300
    gaccagccac actgggactg agacacggcc cagactccta cgggaggcag cagtggggaa 360
    ttttqqacaa tqqqcqaaaq cctqatccaq caatqccqcq tqtqtqaaga aqgccttcgg 420
    qttqtaaaqc acttttgtcc ggaaagaaat ccctqqctct aatacaqtcq qgggatgacg 480
55
    gtaccggaag aataagcacc ggctaactac gtgccagcag ccgcggtaat acgtagggtg 540
    caagogttaa toggaattac toggoogtaaa gogtgogcag goggtttgot aagacogatg 600
```

WO 03/054162 PCT/IIS02/41014 18/52

```
tqaaatcccc qqqctcaacc tgggaactgc attgqtgact ggcaggctag agtatgqcaq 660
    aqqqqqtaq aattccacgt gtagcagtga aatgcgtaga gatgtggagg aataccgatg 720
    qcqaaqqcaq cccctgggc caatactgac gctcatgcac gaaagcgtgg ggagcaaaca 780
    qqattaqata ccctggtagt ccacgcccta aacgatgtca actagttgtt ggggattcat 840
     ttccttagta acgtagctaa cgcgtgaagt tgaccgcctg gggagtacgg tcgcaagatt 900
     aaaactcaaa ggaattgacg gggacccgca caagcggtgg atgatgtgga ttaattcgat 960
    gcaacgcgaa aaaccttacc taccettgac atggteggaa teetgetgag aggtgggagt 1020
    gctcgaaaga gaaccggcgc acaggtgctg catggctgtc gtcagctcgt gtcgtgagat 1080
    gttgggttaa gtcccgcaac gagcgcaacc cttgtcctta gttgctacgc aagagcactc 1140
10
    taaggagact geeggtgaca aaceggagga aggtggggat gaegtcaagt cetcatggee 1200
    cttatgggta gggcttcaca cgtcatacaa tggtcggaac agagggttgc caacccgcga 1260
    gggggagcta atcccagaaa acccatcgta gtccggattg cactctgcaa ctcgagtgca 1320
    tgaagetgga ategetagta ategeggate ageatgeege ggtgaataeg tteeegggte 1380
    ttgtacacac cgcccgtcac accatgggag tgggttttac cagaagtggc tagtctaacc 1440
15
    gcaaggagga cggtcaccac ggtaggattc atgactgggg tgaagtcgta acaaggtagc 1500
    cgtatcggaa ggtgcggctg gatcacctcc tttct
    <210> 42
20
    <211> 1488
     <212> DNA
    <213> Burkholderia mallei
    <400> 42
25
    agattgaacg ctggcggcat gccttacaca tgcaagtcga acggcagcac gggcttcggc 60
    ctggtggcga gtggtgaacg ggtgagtaat acatcggaac atgtcctgta gtgggggata 120
    gcccggcgaa agccggatta ataccgcata cgatctgagg atgaaagcgg gggaccttcg 180
    qqcctcqcqc tataqqqttq qccqatqqct qattaqctaq ttqqtgqqgt aaaggcctac 240
    caaggcgacg atcagtagct ggtctgagag gacgaccagc cacactggga ctgagacacg 300
30
    gcccagactc ctacgggagg cagcagtggg gaattttgga caatgggcgc aagcctgatc 360
    cagcaatgcc gcgtgtgtga agaaggcctt cgggttgtaa agcacttttg tccggaaaga 420
    aatcattctg gctaataccc ggagtggatg acggtaccgg aagaataagc accggctaac 480
    tacgtgccag cagccgcggt aatacgtagg gtgcgagcgt taattggaat tactgggcgt 540
    aaagcgtgcg caggcggttt gctaagaccg atgtgaaatc cccgggctca acctgggaac 600
35
    tgcattggtg actggcaggc tagagtatgg cagagggggg tagaattcca cgtgtagcag 660
    tgaaatgcgt agagatgtgg aggaataccg atggcgaagg cagcccctg ggccaatact 720
    gacgctcatg cacgaaagcg tggggagcaa acaggattag ataccctggt agtccacgcc 780
    ctaaacgatg tcaactagtt gttggggatt catttcctta gtaacgtagc taacgcgtga 840
    agttgaccgc ctggggagta cggtcgcaag attaaaactc aaaggaattg acggggaccc 900
40
    gcacaagcgg tggatgatgt ggattaattc gatgcaacgc gaaaaacctt acctaccctt 960
    gacatggteg gaageeegat gagagttggg egtgetegaa agagaacegg egcacaggtg 1020
    ctgcatggct gtcgtcagct cgtgtcgtga gatgttgggt taagtcccgc aacgagegca 1080
    accettgice tragtigeta egeaagagea etetaaggag actgeeggtg acaaacegga 1140
    ggaaggtggg gatgacgtca agtcctcatg gcccttatgg gtagggcttc acacgtcata 1200
    caatggtegg aacagagggt cgccaacccg cgagggggag ccaatcccag aaaaccgatc 1260
    gtagteegga ttgeactetg caactegagt gcatgaaget ggaategeta gtaategegg 1320
    atcagcatgc cgcggtgaat acgttcccgg gtcttgtaca caccgcccgt cacaccatgg 1380
    gagtgggttt taccagaagt ggctagtcta accgcaagga ggacggtcac cacggtagga 1440
    ttcatgactg gggtgaagtc gtaacaaggt agccgtatcg gaaggtgc
50
```

<210> 43 <211> 1610 <212> DNA

⁵⁵ <213> Burkholderia pseudomallei

```
<400> 43
     tctagatgcg tgctcgagcg gccgcccagt gctgcatgga tatctgctga attcggcttg 60
     agcagtttga teetggetea gattgaaege tggeggeatg cettacacat gcaagtegaa 120
    cggcagcacg ggcttcggcc tggtggcgag tggcgaacgg gtgagttata catcggagca 180
     tgtcctgtag tgggggatag cccggcgaaa gccgaattaa taccgcatac gatctgagga 240
     tgaaageggg ggaeettegg geetegeget atagggttgg eegatggetg attagetagt 300
     tggtggggta aaggcctacc aaggcgacga tcagtagctg gtctgagagg acgaccagcc 360
    acactgggac tgagacacgg cccagactcc tacgggaggc agcagtgggg aattttggac 420
    aatgggegea ageetgatee ageaatgeeg egtgttgtgaa gaaggeette gggttgtaaa 480
10
    gcacttttgt ccggaaagaa atcattctgg ctaatacccg gagtggatga cggtaccgga 540
    agaataaqca ccqqctaact acqtqccaqc aqccqcgqta atacqtaggg tgcgagcgtt 600
    aatcgggatt actgggcgta aagcgtgcgc aggcggtttg ctaagaccga tgtgaaatcc 660
    ccgggctcaa cctgggaact gcattggtga ctggcaggct agagtatggc agagggggt 720
    aqaattccac qtqtaqcagt qaaatqcqta gagatqtgga ggaataccga tggcgaaggc 780
    agcccctgg gccaatactg acgctcatgc acgaaagcgt ggggagaaaa caggattaga 840
    taccetggta gtccacgece taaacgatgt caactagttg ttggggatte atttccttag 900
    taacgtagct aacgegegaa gttgaccgcc tggggagtac ggtcgcaaga ttaaaactca 960
    aaggaattga eggggaceeg cacaageggt ggatgatgtg gattaatteg atgcaaegeg 1020
    aaaaacctta cctacccttg acatggtcgg aagcccgatg agagttgggc gtgctcgaaa 1080
    gagaaccggc gcacaggtgc tgcatggctg tcgtcagctc gtgtcgtgag atgttgggtt 1140
    aagteeegea acgagegeaa eeettgteet tagttgetae geaagageae tetaaggaga 1200
    ctgccggtga caaaccggag gaaggtgggg atgacgtcaa gtcctcatgg cccttatggg 1260
    taqqqcttca cacqtcatac aatggtcgga acagagggtc gccaacccgc gagggggagc 1320
    caatcccaqa aaaccqatcg tagtccggat tgcactctgc aactcgagtg catgaagctg 1380
25
    quategetaq taategegga teageatgee geggtgaata egtteeeggg tettgtacae 1440
    accgcccgtc acaccatggg agtgggtttt accagaagtg gctagtctaa ccgcaaggag 1500
    gacggtcacc acggtaggat tcatgactgg ggtgaagtcg taacaaggta gccgtagaag 1560
    ccgaattcca gcacactggc ggccgttact actggatccg agctcgtacc
30
    <210> 44
     <211> 1544
     <212> DNA
     <213> Neisseria gonorrhoeae
35
     <400> 44
    tgaacataag agtttgatcc tggctcagat tgaacgctgg cggcatgctt tacacatgca 60
     agtcggacgg cagcacaggg aagcttgctt ctcgggtggc gagtggcgaa cgggtgagta 120
     acatategga aegtaeeggg tageggggga taaetgateg aaagateage taataeegea 180
    tacgtcttga gagggaaagc aggggacctt cgggccttgc gctatccgag cggccgatat 240
     ctgattagct ggttggcggg gtaaaggccc accaaggcga cgatcagtag cgggtctgag 300
     aggatgatec gecacactgg gaetgagaca eggeceagac tectaeggga ggeageagtg 360
     gggaattttg gacaatgggc gcaagcctga tccagccatg ccgcgtgtct gaagaaggcc 420
     ttcgggttgt aaaggacttt tgtcagggaa gaaaaggctg ttgccaatat cggcggccga 480
45
     tgacggtacc tgaagaataa gcaccggcta actacgtgcc agcagccgcg gtaatacgta 540
    gggtgcgagc gttaatcgga attactgggc gtaaagcggg cgcagacggt tacttaagca 600
    ggatgtgaaa teeceggget caaceeggga actgegttet gaactgggtg actegagtgt 660
    gtcagaggga ggtggaatte cacgtgtage agtgaaatge gtagagatgt ggaggaatae 720
     cgatggcgaa ggcagcctcc tgggataaca ctgacgttca tgtccgaaag cgtgggtagc 780
50
    aaacaggatt agataccctg gtagtccacg ccctaaacga tgtcaattag ctgttgggca 840
    acttgattgc ttggtagcgt agctaacgcg tgaaattgac cgcctgggga gtacggtcgc 900
    aagattaaaa ctcaaaggaa ttgacgggga cccgcacaag cggtggatga tgtggattaa 960
    ttcqatqcaa cgcgaagaac cttacctggt tttgacatgt gcggaatcct ccggagacgg 1020
    aggaqtgcct tegggageeg taacacaggt getgeatgge tgtegteage tegtgtegtg 1080
55
    agatgttggg ttaagtcccg caacgagegc aaccettgtc attagttgcc atcattcggt 1140
     tgggcactct aatgagactg ccggtgacaa gccggaggaa ggtggggatg acgtcaagtc 1200
```

```
ctcatggccc ttatgaccag ggcttcacac gtcatacaat ggtcggtaca gagggtagcc 1260
     aagccgcgag gcggagccaa tctcacaaaa ccgatcgtag tccggattgc actctgcaac 1320
     togagtgcat gaagtoggaa togotagtaa togoaggtca goatactgcg gtgaatacgt 1380
     tecegggtet totacacace gecegteaca ceatgggagt gggggatace agaagtaggt 1440
     agggtaaccg caaggagtcc gcttaccacg gtatgcttca tgactggggt gaagtcgtaa 1500
     caaqqtaqcc qtaqqqqaac ctqcqqctgg atcacctcct ttct
     <210> 45
10
    <211> 1544
     <212> DNA
     <213> Neisseria meningitidis
     <400> 45
15
    tgaacataag agtttgatcc tggctcagat tgaacgctgg cggcatgctt tacacatgca 60
     agtoggacgg cagcacagag aagottgott otogggtggc gagtggcgaa cgggtgagta 120
     acatategga acqtaecqaq tagtgggga taactgateg aaagateage taatacegea 180
     tacgtcttga gagagaaagc aggggacctt cgggccttgc gctattcgag cggccgatat 240
     ctgattagct agttggtggg gtaaaggcct accaaggcga cgatcagtag cgggtctgag 300
    aggatgatec gecacactgg gactgagaca eggeceagac tectaeggga ggcageagtg 360
    gggaattttg gacaatgggc gcaagcctga tccagccatg ccgcgtgtct gaagaaggcc 420
    ttogggttgt aaaggacttt tgtcagggaa gaaaaggctg ttgctaatat cagcggctga 480
    tgacggtacc tgaagaataa gcaccggcta actacgtgcc agcagccgcg gtaatacgta 540
    gggtgcgagc gttaatcgga attactgggc gtaaagcggg cgcagacggt tacttaagca 600
    ggatgtgaaa tccccgggct caacccggga actgcgttct gaactgggtg actcgagtgt 660
    qtcaqaqqa gqtaqaattc cacqtgtaqc agtgaaatgc gtagagatgt ggaggaatac 720
     cqatqqcqaa qqcaqcctcc tqqqacaaca ctqacqttca tqcccqaaag cqtqqqtaqc 780
     aaacaggatt agataccctg gtagtccacg ccctaaacga tgtcaattag ctgttgggca 840
    acctgattgc ttggtagcgt agctaacgcg tgaaattgac cgcctgggga gtacggtcgc 900
30
     aagattaaaa ctcaaaggaa ttgacgggga cccgcacaag cggtggatga tgtggattaa 960
    ttogatgcaa cgcgaagaac cttacctggt cttgacatgt acggaatcct ccggagacgg 1020
     aggagtgeet tegggageeg taacacaggt getgeatgge tgtegteage tegtgtegtg 1080
     agatgttggg ttaagtcccg caacgagcgc aaccettgte attagttgcc atcattcagt 1140
    tgggcactct aatgagactg ccggtgacaa gccggaggaa ggtggggatg acgtcaagtc 1200
35
    ctcatqqccc ttatqaccaq qqcttcacac qtcatacaat qqtcqgtaca qaqqqtaqcc 1260
    aagccgcgag gcggagccaa tctcacaaaa ccgatcgtag tccggattgc actctgcaac 1320
     tegagtgeat gaagteggaa tegetagtaa tegeaggtea geatactgeg gtgaataegt 1380
     tcccgggtct tgtacacacc gcccgtcaca ccatgggagt gggggatacc agaagtaggt 1440
     aggataacca caaggagtcc gcttaccacg gtatgcttca tgactggggt gaagtcgtaa 1500
40
    caaqqtaqcc gtaqqqqaac ctgcqqctqq atcacctcct ttct
     <210> 46
     <211> 1537
45
     <212> DNA
     <213> Pseudomonas aeruginosa
    <400> 46
    gaactgaaga gtttgatcat ggctcagatt gaacgctggc agcaggggcc ttcaacacat 60
    gcaagtcgag cttatgaagg gagcttgcct tggattcagc ggcggacggg tgagtaatgc 120
    ctaggaatet gcctggtagt gggggataac gtccggaaac ggccgctaat accgcatacg 180
    tcctgaggga gaaagtcggg gatcttcgga cctcacgcta tcagatgagc ctaggtcgga 240
    ttagctagtt ggtggggtaa aggcctacca aggcgacgat ccgtaactgg tctgagagga 300
    tqatcaqtca cactqqaact gagacacggt ccaqactcct acqqqaqqca qcaqtqqqqa 360
    atattqqaca atgggcgcaa gcctgatcca gccatgccgc gtgtgtgaag aaggtcttcg 420
    gattqtaaaq cactttaagt tgggaggaag ggcagtaagt taataccttg ctgtttgacg 480
```

WO 03/054162 PCT/US02/41014 21/52

```
ttaccaacag aataagcacc ggctaacttc gtgccagcag ccgcggtaat acgaagggtg 540
    caagegttaa teggaattae tgggegtaaa gegegegtaa gtggtteage aagettgatg 600
    tgaaatcccc gggctcaacc tgggaactgc atccaaaagc tactgagcta gagtacggta 660
    gaggtggtag aatttcctgt gtagcggtga aatgcgtaga tataggaagg aacaccagtg 720
    gcgaaggcga ccacctggac tgtactgaca ctgaggtgcg aaagcgtggg gagcaaacag 780
    gattagatac cotqqtaqto cacqccqtaa acqatqtcqa ctaqccqttq qgatccttqa 840
    gatettagtg gegeaegtaa egegataagt egacegeetg gggagtaegg eegeaaggtt 900
    aaaactcaaa tqaattgacg ggggcccgca caagcggtgg agcatgtggt ttaattcgaa 960
    gcaacqcqaa gaaccttacc tggccttgac atgctgagaa ctttccagag atggattggt 1020
10
    gccttcggga acagagacac aggtgctgca tggctgtcgt cagctcgtgt cgtgagatgt 1080
    toggttaagt cccqtaacqa qcqcaaccct tqtccttaqt taccaqcacc tcqqqtqqqc 1140
    actictaagga gactgooggt gacaaaccgg aggaaggtgg ggatgacgtc aagtcatcat 1200
    qqcccttacq qccaqqqcta cacacqtqct acaatqqtcq qtacaaaqqq ttqccaagcc 1260
    gcgagtggga gctaatccca taaaaccgat cgtagtccgg atcgcagtct gcaactcgac 1320
15
    tgcgtgaagt cggaatcgct agtaatcgtg aatcagaatg tcacggtgaa tacgtccccg 1380
    ggccttgtac acacegcccg tcacaccatg ggagtgggtt gctccagaag tagctagtct 1440
    aaccgcaagg gggacggtta ccacggagtg attcatgact ggggtgaagt cgtaacaagg 1500
    tagecgtagg ggaacetgeg getggateae eteetta
20
     <210> 47
     <211> 1467
     <212> DNA
     <213> Vibrio cholerae
25
    <220>
     <221> modified base
    <222> (928) . . (1464)
    <223> N = A, C, G or T/U
30
    <400> 47
    attgaagagt ttgatcctgg ctcagattga acgctggcgg caggcctaac acatgcaagt 60
    cqaqcqqcaq cacaqaqqaa cttgttcctt gggtggcgag cggcggacgg gtgagtaatg 120
    cctgggaaat tgcccggtag agggggataa ccattggaaa cgatggctaa taccgcataa 180
35
    cctcgcaaqa gcaaagcagg ggaccttcgg gccttgcgct accggatatg cccaggtggg 240
    attagctagt tggtgaggta agggctcacc aaggcgacga tccctagctg gtctgagagg 300
    atgatcagcc acactggaac tgagacacgg tccagactcc tacgggaggc agcagtgggg 360
    aatattqcac aatqqccqca aqcctqatgc aqccatgccq cgtgtatgaa qaaqqccttc 420
    gggttgtaaa gtactttcag tagggaggaa ggtggttaag ttaatacctt aatcatttga 480
40
    cgttacctac agaagaagca ccggctaact ccgtgccagc agccgcggta atacggaggg 540
    tgcaagcgtt aatcggaatt actgggcgta aagcgcatgc aggtggtttg ttaagtcaga 600
    tgtgaaagcc ctgggctcaa cctaggaatc gcatttgaaa ctgacaagct agagtactgt 660
    aqagggggt agaatttcag gtgtagcggt gaaatgcgta gagatctgaa ggaataccgg 720
    tgqcgaaggc ggcccctgg acagatactg acactcagat gcgaaagcgt ggggagcaaa 780
45
    caggattaga taccetggta gtecacgeeg taaacgatgt ctacttggag gttgtgccct 840
    agagtegtgg cttteggage taaegegtta agtagaeege etggggagta eggtegeaag 900
    attaaaactc aaatgaattg acggggncc gcacaagcgg tggagcatgt ggtttaattc 960
    ganncaacgc gaagaacctt acctactctt gacatccaga gaatctagcg gagacgctgg 1020
    agtgccttcg ggagctctga gacaggtgct gcatggctgt cgtcagctcg tgttgtgaaa 1080
50
    tgttgggtta agtcccgcaa cgagcgcaac ccttatcctt gtttgccagc acgtaatggt 1140
    gggaactcca gggagactgc cggtgataaa ccggaggaag gtggggacga cgtcaagtca 1200
    tcatggccct tacgagtagg gctacacacg tgctacaatg gcgtatacag agggcagcga 1260
    taccgcgagg tggagcgaat ctcacaaagt acgtcgtagt ccggattgga gtctgcaact 1320
    cgactccatg aagtcggaat cgctagtaat cgcaaatcag aatgttgcgg tgaatacgtt 1380
55
    cccqqqcctt gtacacaccg cccgtcacac catgggagtg ggctgcaaaa gaagcangta 1440
    gtttaacctt cgggaggacg cttnccc
```

```
<210> 48
    <211> 1485
    <212> DNA
    <213> Yersinia enterocolitica
    <220>
    <221> modified base
10 <222> (1)..(1484)
    <223> N = A, C, G or T/U
    <400> 48
    naattqaaqa qtttqatcat ggctcagatn gaacgctggc ggcaggccta acacatgcaa 60
    gtcgagcggc agcgggaagn agtttactac tttcngggcg agcggcgnac gggtgagtaa 120
    tgtctgggaa actgcctgat ggagggggat aactactgga aacggtagct aataccgcat 180
    aacgtetteg gaccaaagtg ggggacetta gggcetcacg ccatengatg tgcccagatg 240
    ggattageta gtaggtgggg taatggetca cetaggegac gatecetage tggtetgaga 300
    ggatgaccag ccacactgga actgagacac ggtccagact cctacgggag gcagcagtgg 360
20
   ggaatattgc acaatgggcg caagcctgat gcagccatgc cgcgtgtgtg aagaaggcct 420
    togggttgta aagcactttc agcgaggagg aaggccaata acttaatacg ttgttggatt 480
    qacqttactc qcaqaaqaaq caccqqctaa ctccqtqcca gcaqccqcqg taatacqqag 540
    ggtgcaagcg ttaatcggaa ttactgggcg taaagcgcac gcaggcggtt tgttaagtca 600
    qatqtqaaat ccccqcqctt aacgtgggna cngcatttga aactggcaag ctagagtctt 660
    gtagaggggg gtagaattcc aggtgtagcg gtgaaatgcg tagagatctg naggaatacc 720
25
    ggtggcgaag gcggccccct ggacaaagac tgacgctcag gtgcgaaagc gtggggagca 780
    aacaggatta gataccetgg tagtecacge tgtaaacgat gtegacttgg aggttgtgcc 840
    cttgaggegt ggetteegga getaaegegt taagtegaee geetggggag taeggeegea 900
    aggttaaaac tcaaatgaat tnncgggggc cngcacaagc ggtggagcat gtggtttaat 960
30 togatqcaac qcqaaqaacc ttacctactc ttgacatcca cggaatttag cagagatgct 1020
    ttagtgnctt cgggaaccgt gagacaggtg ctgcatggct gtcgtcagct cgtgttgtga 1080
    aatqttqqqt taaqtcccqc aacqaqcqca accettatec tttgttqcca gcacqtaatg 1140
    gtgggaactc aaaggagact gccggtgata aaccggagga aggtggggat gacgtcaagt 1200
    catcatggcc cttacgagta gggctacaca cgtgctacaa tggcagatac aaagtgaagc 1260
35
    gaactcgcga gagcaagcgg accacataaa gtctgtcgta gtccggattg gagtctgcaa 1320
    ctcgactcca tgaagtcgga atcgctagta atcgtagatc agaatgctac ggtgaatacg 1380
    ttcccgggcc ttgtacacac cgcccgtcac accntgggag tgggttgcaa aagaagtagg 1440
    tagettaaen ttegggaggg egegtaeeae tttgtgatte nngne
40
    <210> 49
    <211> 2927
    <212> DNA
    <213> Bacillus subtilis
45
    <400> 49
    ggttaagtta gaaagggcgc acggtggatg ccttggcact aggagccgat gaaggacggg 60
    acgaacaccg atatgetteg gggagetgta agcaagettt gateeggaga ttteegaatg 120
    gggaaaccca ccactcgtaa tggagtggta tccatatctg aattcatagg atatgagaag 180
50
    gcagaccegg ggaactgaaa catctaagta ceeggagaag agaaagcaaa tgcgatteec 240
    tgagtagegg cgacgaacac gggatcagec caaaccaaga ggettgeete tgtggttgta 300
    qqacactctq tacqqaqtta caaaaqaacq aqqtagatqa aqaqqtctgg aaagggcccg 360
    ccataggagg taacagccct gtagtcaaaa cttcgttctc tcctgagtgg atcctgagta 420
    cggcggaaca cgtgaaattc cgtcggaatc cgggaggacc atctcccaag gctaaatact 480
    ccctagtgac cgatagtgaa ccagtaccgt gagggaaagg tgaaaagcac cccggaaggg 540
    gagtgaaaga gatcctgaaa ccgtgtgcct acaagtagtc agagcccgtt aacggtgatg 600
```

```
gegtgeettt tgtagaatga accggegagt tacgateceg tgcaaggtta agcagaagat 660
    gcggagccgc agcgaaagcg agtctgaata gggcgcatga gtacgtggtc gtagacccga 720
    aaccaggtga totacccatg tocagggtga agttcaggta acactgaatg gaggcccgaa 780
    cccacgcacg ttgaaaagtg cggggatgag gtgtgggtag gggtgaaatg ccaatcgaac 840
    ctggagatag ctggttctct ccgaaatagc tttagggcta gcctcaaggt aagagtcttg 900
    gaggtagage actgattgga ctaggggcc tcaccgggtt accgaattca gtcaaactcc 960
    quatqccaat qacttatect tqqqaqtcaq actqcqaqtq ataaqatecq taqtcqaaaq 1020
    qqaaacaqcc cagaccgcca qctaaqqtcc caaagtatac gttaaqtgga aaagqatqtg 1080
    qaqttqctta gacaaccaqq atgttqqctt agaagcagcc accatttaaa gaqtqcqtaa 1140
10
    tageteactg gtegagtgae tetgegeega aaatgtaceg gggetaaaeg tateacegaa 1200
    gctgcggact gttcttcgaa cagtggtagg agagcgttct aagggctgtg aagccagacc 1260
    ggaaggactg gtggacggct tagaagtgag aatgccggta tgagtagcga aaagagggt 1320
    gagaatccct ccaccgaatg cctaagggtt cctgaggaag gctcgtccgc tcagggttag 1380
    togggaccta agcogaggo gaaaggogta ggcgatggac aacaggttga tattoctgta 1440
15 ccacctcctc accatttgag caatgggggg tcgcaggagg atagggtaag cgcggtattg 1500
    qatatccqcq tccaaqcaqt tagqctggqa aataqgcaaa tccgtttccc ataaqqctqa 1560
    qctqtqatqq cqaqcqaaat ataqtaqcqa agttcctgat tccacactgc caaqaaaaqc 1620
    ctctagcgag gtgagaggtg cccgtaccgc aaaccgtcac aggtaggcga ggagagaatc 1680
    ctaaqqtqat cgaqagaact ctcqttaaqq aactcqqcaa aatgaccccq taacttcqqq 1740
20
    agaaggggtg ctctgttagg gtgcaagccc gagagagccg cagtgaatag gcccaggcga 1800
    ctgtttagca aaaacacagg tctctgcgaa gccgtaaggc gaagtatagg ggctgacgcc 1860
    tocccqqtqc tqqaaqqtta aqaqqaqcqc ttaqcqtaaq cqaaqqtqcq aattqaaqcc 1920
    ccaqtaaacq qcqqccqtaa ctataacqqt cctaaqqtaq cqaaattcct tqtcqqqtaa 1980
    gttccgaccc gcacgaaagg cgcaacgatc tgggcgctgt ctcaacgaga gactcggtga 2040
25
    aattataqta cctqtqaaqa tqcaqqttac ccqcqacaqq acqqaaagac cccqtqqaqc 2100
    tttactqcaq cctqatattq aatgttqqta caqcttqtac aggataggta ggagccttqq 2160
    aaaccggagc gccagcttcg gtggaggcat cggtgggata ctaccctggc tgtattgacc 2220
    ttctaacccc ccgcccttat cgggcgggga gacagtgtca ggtgggcagt ttgactgggg 2280
    cgqtcgcctc ctaaaaggta acggaggcgc ccaaaggttc cctcagaatg gttggaaatc 2340
    attogcagag tgtaaaggca caagggagct tgactgcgag acctacaagt cgagcaggga 2400
    cgaaagtcgg gcttagtgat ccggtggttc cgcatggaag ggccatcgct caacggataa 2460
    aagctacccc ggggataaca ggcttatctc ccccaagagc tccacatcga cggggaggtt 2520
    tggcacctcg atgtcggctc atcgcatcct ggggctgtag tcggtcccaa gggttgggct 2580
    qttcqcccat taaaqcqqta cqcqaqctqq qttcaqaacq tcqtqaqaca qttcqqtccc 2640
35
    tatccqtcqc qqqcqctqqa aatttqaqaq qaqctqtcct tagtacgaga gqaccgggat 2700
    ggacgcaccg ctggtgtacc agttgttctg ccaagggcat cgctgggtag ctatgtgcgg 2760
    acgggataag tgctgaaagc atctaagcat gaagcccccc tcaagatgag atttcccatt 2820
    ccgcaaggaa gtaagatccc tgaaagatga tcaggttgat aggtctgagg tggaagtgtg 2880
    gcaacacatg gagctgacag atactaatcg atcgaggact taaccat
40
    <210> 50
    <211 > 2922
    <212> DNA
45
    <213> Bacillus anthracis
    <400> 50
    ggttaagtta gaaagggcgc acggtggatg ccttgacact aggagtcgat gaaggacggg 60
    actaacgccg atatgcttcg gggagctgta agtaagcttt gatccgaaga tttccgaatg 120
50
    gqgaaaccca ccatacgtaa tggtatggta tccttatctg aatacatagg gtaaggaaga 180
    cagacccagg gaactgaaac atctaagtac ctggaggaag agaaagcaaa tgcgatttcc 240
    tgagtagcgg cgagcgaaac ggaacatagc ccaaaccaag aggcttgcct cttggggttg 300
    taggacattc tatacggagt tacaaaggaa cgaggtagac gaagcgacct ggaaaggtcc 360
    qtcqtaqaqq qtaacaaccc cqtaqtcqaa acttcqttct ctcttqaatq tatcctqaqt 420
    acggcggaac acgtgaaatt ccgtcggaat ctgggaggac catctcccaa ggctaaatac 480
    tecctagtqa tegatagtqa accagtaceq tqagggaaaq qtqaaaagca ceceggaagg 540
```

55

```
ggagtgaaag agatcctgaa accgtgtgcc tacaaatagt cagagcccgt taacgggtga 600
     tggcgtgcct tttgtagaat gaaccggcga gttacgatcc cgtgcgaggt taagctgaag 660
     aggoggagec geagegaaag egagtetgaa tagggegttt agtacgtggt egtagacecg 720
     aaaccaggtg atctacccat gtCcagggtg aagttcaggt aacactgaat ggaggcccga 780
    acccacgcac gttgaaaagt gcggggatga ggtgtgggta gcggagaaat tccaatcgaa 840
     cctggagata gctggttctc cccgaaatag ctttagggct agccttaagt gtaagagtct 900
     tggaggtaga gcactgattg gactaggggt cctcatcgga ttaccgaatt cagtcaaact 960
     ccqaatqcca atqacttatc cttaggagtc aqactqcqaq tqataaqatc cqtaqtcaaa 1020
     agggaaacag cccagaccgc cagctaaggt cccaaagtgt gtattaagtg gaaaaggatg 1080
10 tggagttgct tagacaacta ggatgttggc ttagaagcag ccaccattta aagagtgcgt 1140
    aatageteae tagtegagtg actetgegee gaaaatgtae eggggetaaa tacaccaeeg 1200
     aagctgcgga ttgataccaa tggtatcagt ggtaggggag cgttctaagg acagtgaagt 1260
    cagaceggaa ggactggtgg agtgcttaga agtgagaatg ceggtatgag tagegaaaga 1320
    cgggtgagaa tcccgtccac cgaatgccta aggtttcctg aggaaggctc gtccgctcag 1380
15 ggttagtcag gacctaagcc gaggccgaca ggcgtaggcg atggacaaca ggttgatatt 1440
     cctgtaccac ctctttatcg tttgagcaat ggagggacgc agaaggatag aagaagcgtg 1500
     cgattggttg tgcacgtcca agcagttagg ctgataagta ggcaaatccg cttatcgtga 1560
     aggotgagot gtgatgggga agotoottat ggagogaagt otttgattoo cogotgocaa 1620
    gaaaagcttc tagcgagata aaaggtgcct gtaccgcaaa ccgacacagg tagqcgagga 1680
    gagaatccta aggtgtgcga gagaactctg gttaaggaac tcggcaaaat gaccccgtaa 1740
    cttcgggaga aggggtgctt tcttaacgga aagccgcagt gaataggccc aagcgactgt 1800
    ttagcaaaaa cacagctctc tgcgaagccg taaggcgaag tatagggggt gacacctgcc 1860
    cggtgctgga aggttaagga gaggggttag cgtaagcgaa gctctgaact gaagccccag 1920
    taaacggcgg ccgtaactat aacggtccta aggtagcgaa attccttgtc gggtaagttc 1980
25 cgacccgcac gaaaggtgta acgatttggg cactgtctca accagagact cggtgaaatt 2040
    atagtacctg tgaagatgca ggttacccgc gacaggacgg aaagaccccg tggagcttta 2100
    ctgtagcctg atattgaatt ttggtacagt ttgtacagga taggcgggag cctttgaaac 2160
    cggagcgcta gcttcggtgg aggcgctggt gggataccgc cctgactgta ttgaaattct 2220
    aacctacggg tettategae eegggagaea gtgteaggtg ggeagtttga etggggeggt 2280
30
   cgcctcctaa agtgtaacgg aggcgccaa aggttcctc agaatggttg gaaatcattc 2340
    gtagagtgca aaggcataag ggagcttgac tgcgagacct acaagtcgag cagggacgaa 2400
    agtoggett agtgatoogg tggttoogca tggaagggcc atogctcaac ggataaaagc 2460
    tacccegggg ataacaggct tatctcccc aagagtccac atcgacgggg aggtttggca 2520
    cctcgatgtc ggctcatcgc atcctggggc tgtagtcggt cccaagggtt gggctgttcg 2580
35 cccattaaag cggtacgcga gctgggttca gaacgtcgtg agacagttcg gtccctatcc 2640
    gtcgtgggcg taggaaattt gagaggagct gtccttagta cgagaggacc gggatggacg 2700
    caccgctggt gtaccagttg ttctgccaag ggcatagctg ggtagctatg tgcggaaggg 2760
    ataagtgctg aaagcatcta agcatgaagc cccctcaag atgagatttc ccatagcgta 2820
    agctagtaag atccctgaaa gatgatcagg ttgataggtt cgaggtggaa gcatggtgac 2880
40
    atgtggagct gacgaatact aatagatcga ggacttaacc at
    <210> 51
    <211> 2912
45
    <212> DNA
    <213> Enterococcus faecalis
    <400> 51
    ggttaagtga ataagggcgc acggtggatg ccttggcact aggagccgat gaaggacggg 60
    actaacaccg atatgctttg gggagctgta agtaagctat gatccagaga tttccgaatg 120
```

ggggaaccca atactittta taggitatta citticaggi atacatage tgattagag 180 tagacgcaga gaactgaasa stettagitac citgagggaa agaasgaasa titcgattoc 240 tgagitagcgg cgagcgaasa gggaaagagc caaaccaaca agctigctig tiggggitgi 300 aqqactocsa batqutagit cittagitat qitqagaqai tiqqaaaati coqciaaaga 360

gggtgaaagc cccgtagacg aaatgctaac aacacctagg aggatcctga gtacggcgga 420 acacgagaaa ttccgtcgga atccgcgggg accatcccgc aaggctaaat actccctagt 480

```
gaccgatagt gaaccagtac cgtgagggaa aggtgaaaag cacccgggaa ggggagtgaa 540
     atagatectg aaaccgtgtg cctacaacaa gtcaaagete gttaatgagt gatggcgtgc 600
     cttttgtaga atgaaccggc gagttacgat tgcatgcgag gttaagtcga agagacggag 660
     ccgcagcgaa agcgagtctg aatagggcga atgagtatgt agtcgtagac ccgaaaccat 720
     gtgatctacc catgtccagg ttgaaggtgc ggtaaaacgc actggaggac cgaacccacg 780
     tacgttgaaa agtgcgggga tgaggtgtgg gtagcggaga aattccaaac gaacttggag 840
     atagctggtt ctctccgaaa tagctttagg gctagcctcg gaattgagaa tgatggaggt 900
     agagcactgt ttggactagg ggcccatctc gggttaccga attcagataa actccgaatg 960
     ccattcattt atatccggga gtcagactgc gagtgataag atccgtagtc gaaagggaaa 1020
10
     cagcccagac caccagctaa ggtcccaaaa tatatgttaa gtggaaaagg atgtgggtt 1080
     gcacagacaa ctaggatgtt ggcttagaag cagccaccat ttaaagagtg cgtaatagct 1140
     cactagtoga gtgaccotgo googaaaatg tacogggot aaacatatta cogaagctgt 1200
     qqactacacc attaqqtqta qtqqtaqqaq aqcqttctaa qqqcqttqaa qqtcqatcqt 1260
     gaggacgct ggagcgctta gaagtgagaa tgccggtatg agtagcgaaa gacaggtgag 1320
15
     aatcctqtcc accgtatqac taaggtttcc tqqqqaaqqc tcqtccqccc aqqqttaqtc 1380
     gggacctaag ccgaggccga taggcgtagg cgatggacaa caggttgata ttcctgtacc 1440
     agttgttttt gtttgagcaa tggagggacg cagtaggcta aggaatgcat gcgattggaa 1500
     gtgcatgtcc aagcaatgag tottgagtag agttaaatgc tttactcttt aaggacaagt 1560
     tgtgacgggg agcgaaataa tagtagcgaa gttcctgatg tcacactgcc aagaaaagct 1620
20
     tctagtgaga aaacaactgc ccgtaccgta aaccgacaca ggtagtcgag gagagtatcc 1680
     taaggtgagc gagcgaactc tcgttaagga actcggcaaa atgaccccgt aacttcggga 1740
     gaaggggtgc tgacttcggt cagccgcagt gaataggccc aagcgactgt ttatcaaaaa 1800
     cacaggtete tgcaaaateg taagatgaag tatagggget gacgeetgee eggtgetgga 1860
     aggttaagag gatgggttag cttcggcgaa gctcagaatt gaagccccag taaacggcgg 1920
25 ccgtaactat aacggtccta aggtagcgaa attccttgtc gggtaagttc cgacccgcac 1980 gaaaggcgta acgatttggg cactgtctca acggagaact cggtgaaatt ttagtacctg 2040
     tgaagatgca ggttacccgc gacaggacgg aaagacccca tggagcttta ctgtagtttg 2100
     atattgagtg tttgtaccac atgtacagga taggtaggag ccgatgagac cggaacgcta 2160
    gtttcggagg aggcgctggt gggatactac ccttgtgtta tgaaccctct aacccgcacc 2220
30
    actaatcgtg gtgggagaca gtgtcagatg ggcagtttga ctggggcggt cgcctcctaa 2280
     aaggtaacgg aggcgcccaa aggttccctc agaatggttg gaaatcattc gaagagtgta 2340
     aaggcagaag ggagcttgac tgcgagacct acaagtcgag cagggacgaa agtcgggctt 2400
     agtgatccgg tgqttccgca tggaagggcc atcqttcaac ggtaaaagct accttgggga 2460
     taacaggett ateteeccca agagtecaca tegacgggga ggtttggcac etegatgteg 2520
35 octogtogca tootggggct gtagtoggto ccaagggttg ggotgttogc ccattaaagc 2580
     ggcacgcgag ctgggttcag aacgtcgtga gacagttcgg tccctatccg tcgcgggcgt 2640
     tggaaatttg agaggagctg tccttagtac gagaggaccg ggatggactt accgctggtg 2700
     aagcatctaa qtqtqaaqcc cacctcaaqa tqaqatttcc catttcttta aqaaaqtaaq 2820
40
    acccctgaga gatgatcagg tagataggtt ggaagtggaa ggctagtgat agttggagcg 2880
     gaccaatact aatcggtcga ggacttaacc aa
```

```
<210> 52
45 <211> 2898
<212> DNA
```

<213> Lactococcus lactis

<a href="color: red;
 <a href="color: red;
 <

WO 03/054162 PCT/IIS02/41014 26/52

```
gcqaaatcca gtttgaatcc gggaggacca tctcccaacc ctaaatactc cttagtgacc 480
    gataqtqaac cagtaccgtg agggaaaggt gaaaagaacc cgagagggga gtgaaatagc 540
    acctgaaacc gtgtgcctac aagaagttcg agcccgttaa tgggtgagag cgtgcctttt 600
    gtagaatgaa ccggcgagtt acgttatgat gcgaggttaa gttgaagaga cggagccgta 660
    gggaaaccga gtctgaatag ggcgacttag tatcatgatg tagacccgaa acctagtgac 720
    ctatccatga gcagggtgaa ggtgtggtaa gacgcactgg aggcccgaac caggacacgt 780
    tqaaaaqtqt ttqqatqact tqtqqataqc qqaqaaattc caaacqaact gggaqataqc 840
    tggttctctc cgaaatagct ttagggctag cgtcgaaatg taagtgtatt ggaggtagag 900
    cactgtttgg gtgaggggtc cgtctaggat taccaatctc agataaactc cgaatgctaa 960
10
    tacacatqtt cqqcaqtcaq actgcqaqtq ctaagatccq tagtcqaaaq ggaaacagcc 1020
    caqaccaaca gctaaggtcc caaaatatat gttaagtgga aaaggatgtg gggttgcaca 1080
    gagaactagg atgttagctc agaagcagct atcattcaaa gagtgcgtaa tagctcacta 1140
    gtcgagtgac cctgcgccga aaatgtaccg gggctaaaca tattaccgaa gctttggatt 1200
    gatattttat caatggtagg agagggttet taaccgcgat gaaggtatac cgtgaggagt 1260
    gctggagcgt taagaagtga gaatgccggt atgagtagcg caagataagt gagaatctta 1320
    tccaccgtaa gactaaggtt tccaggggaa ggctcgtccg ccctgggtta gtcgggacct 1380
    aaggcgaggc cgaaaggcgt agtcgatgga caactggttg atattccagt actagatatg 1440
    atcgtgatgg agggacgcag taggctaaga gatgccagtt aatggattct ggtctaagca 1500
    gtgaggtgtg agatgtgtca aatgcatttc tctttaacat tgagctgtga tggggaagca 1560
20
    actacggttg cgaactctct gatgtcacac tgccaagaaa agcttctagc gtaaagtcat 1620
    atctacccgt accgcaaacc gacacaggtg gtcgaggcga gtagcctcag gtgatcgaga 1680
    gaactetegt taaggaacte ggcaaaatag ceeegtaact tegggagaag gggtgetggt 1740
    gtaaaagcca gccgcagtga ataggcccaa gcaactgttt atcaaaaaca cagctctctg 1800
    ctaaaccgca aggtgatgta tagggggtga cgcctgcccg gtgctggaag gttaagagga 1860
25
    gtgcttagac gtaagtcgaa ggtatgaatt gaagccccag taaacggcgg ccgtaactat 1920
    aacggtccta aggtagcgaa attccttgtc gggtaagttc cgacccgcac gaaaggcgta 1980'
    atgatttggg cactgtctca acgagagact cggtgaaatt ttagtacctg tgaagatgca 2040
    qqttacccgc gacaggacgg aaagacccca tggagcttta ctgtagtttg atattgagta 2100
    cctgtaagtc atgtacagga taggtaggag ccattgaaat agggacgcta gtttctattg 2160
    aggegttgtt gggatactac ccttgactta tggttactct aaccegctgg cataatcggc 2220
    cagggagaca gtgtctgacg gacagtttga ctggggcggt cgctcctaaa gagtaacgga 2280
    ggcgctcaaa ggttggctca gattggttgg aaatcaatcg tagagtgtaa aggtaaaagc 2340
    cagcttgact gcgagagcta caactcgagc aggtaggaaa ctaggactta gtgatccggt 2400
    qqtaccqcat qqaaqqqcca tcgctcaacg gataaaagct accctgggga taacaggctt 2460.
35
    atctcccca agagttcaca tcgacggga ggtttggcac ctcgatgtcg gctcgtcgca 2520
    tcctggggct gtagtcggtc ccaagggttg ggctgttcgc cattaaagcg gcacgcgagc 2580
    tgggttcaga acgtcgtgag acagttcggt ccctatccgt cgcgggcgta ggtaatttga 2640
    gaggatetgt cettagtacg agaggacegg gatggaetta eegetggtgt accagttgtt 2700
    ccgccaggag cacggctgga tagctatgta gggaagggat aagcgctgaa agcatctaag 2760
40
    tgcgaagccc acctcaagat gagattaccc attcgtaaga attaagagcc cagagagatg 2820
    atctggtaga taggctggaa gtggaagagt tgcgagactt ggagcggacc agtactaatc 2880
    gctcgaggac tttaccaa
45
    <210> 53
    <211> 2932
    <212> DNA
    <213> Listeria monocytogenes
50
    <400> 53
    ggttaagtta gaaagggcgc acggtggatg ccttggcact aggagccgaa gaaggacggg 60
    actaacaccg atatgetttg gggagetgta egtaagegtt gatecagaga tttccgaatg 120
    qqqqaaccca ctatctttag tcqqataqta tccttacqtg aatacatagc gtgaggaagg 180
```

caqacccaqq qaactgaaac atctaagtac ctggaggaag agaaagaaaa atcgatttcc 240 tgagtagcgg cgagcgaaac ggaaagagcc caaaccaaga agcttgcttc ttggggttqt 300 aggacactct atacggagtt acaaaagaaa gttataaatg aagcggtctg gaaaggcccg 360 27/52

	ccaaagacgg	taacagcccg	gtagttgaaa	tggctttccc	tccagagtgg	atcctgagta	420
	cgqcqqaaca	cgtgaaattc	cgtcggaatc	cgggaggacc	atctcccaag	gctaaatact	480
	ccctaqtqac	cqataqtgaa	ccagtaccgt	gagggaaagg	tgaaaagcac	cccggaaggg	540
	qaqtqaaaca	gttcctgaaa	ccgtgtgcct	acaagtagtt	agagcccgtt	aatgggtgat	600
5	agcgtgcctt	ttgtagaatg	aaccggcgag	ttacgatttg	ttgcaaggtt	aagcggaaaa	660
	agcggagccg	tagcgaaagc	gagtctgaat	agggcgcata	agtaacaggt	cgtagacccg	720
	aaaccaggtg	atctacccat	gtccaggatg	aaggtaaggt	aatacttact	ggaggtccga	780
	acccacgcac	gttgaaaagt	gcggggatga	ggtgtgggta	gcggagaaat	tccaatcgaa	840
	cttggagata	gctggttctc	tccgaaatag	ctttagggct	agcctcgagg	taaagagtca	900
10	tggaggtaga	gcactgtttg	gactaggggc	ccttctcggg	ttaccgaatt	cagataaact	960
	ccgaatgcca	tgtacttata	ctcgggagtc	agactgcgag	tgataagatc	cgtagtcgaa	1020
						gaaaaggatg	
						aagagtgcgt	
						catattaccg	
15	aaactgtgga	tgaacctctt	tagaggttcg	tggtaggaga	gcgttctaag	ggcggtgaag	1260
	tcagaccgga	aggactggtg	gagcgcttag	aagtgagaat	gccggtatga	gtagcgaaag	1320
	aagggtgaga	atcccttcca	ccgaatatct	aaggtttcct	gaggaaggct	cgtccgctca	1380
	gggttagtcg	ggacctaagc	cgaggccgat	aggcgtaggc	gatggacaac	aggtagagat	1440
	tcctgtacca	gtgctaattg	tttaaccgat	ggggtgacac	agaaggatag	ggaatcgcac	1500
20	gaatggaaat	gtgcgtccaa	gcagtgagtg	tgagaagtag	gcaaatccgc	ttctcacgaa	1560
	gcatgagctg	tgatggggaa	ggaaattaag	tacggaagtt	cctgatttca	cgctgtcaag	1620
	aaaagcctct	aggaagagta	gtactgcccg	taccgcaaac	cgacacaggt	agatgaggag	1680
						accccgtaac	
0.5	ttcgggagaa	ggggtgctct	attagggtgc	aagcccgaga	gagccgcagt	gaataggccc	1800
25	aggcgactgt	ttagcaaaaa	cacaggtete	tgcaaaaccg	taaggtgacg	tataggggct	1000
	gacgcctgcc	cggtgctgga	aggttaagag	gagtgettag	cttcggcgaa	ggtacgaatt	1000
						attccttgtc	
	gggtaagttc	cgacccgcac	gaaaggegea	acgatcuggg	cactgtetta	acgagagact	2100
30	cggtgaaatt	atagtacctg	tgaagatgca	ggttaccege	gacaggacgg	aaagaccccg	2100
30	tggagcttta	ctgcaacctg	acataggaacg	agggaatagt	ccgcacagga	taggtaggag cctggctgta	2220
	ccgaagagac	gegegeeea	geatacgagg	tagagagaga	gtgtcaccac	ggcagtttga	2280
	atagggggg	accedecac	agagtaagag	agggagaca	aggttggtg	agaatggatg	2340
	gazatoatto	gagagagaga	agagcaacag	aggegeeeaa	tacasaseta	acaagtcgag	2400
35	gaaaccaccc	agtegggett	adgeteed	tagttccaca	tagaagagac	atcgctcaac	2460
55	ggatgaa	taccccaaaa	ataacaggct	tatetecce	aagagtccac	atcgacgggg	2520
	agatttagge	cctccatctc	aactcatcac	atcctggggc	tataatcaat	cccaagggtt	2580
	aggeteggea	cccattagge	caacacacaa	actagattca	gaacgtcgtg	agacagttcg	2640
	gtccctatcc	atcacaaaca	caggaaattt	gagaggaget	gtccttagta	cgagaggacc	2700
40	gagatagaca	caccactaat	graccagttg	ttccgccagg	agcatcgctg	ggtagctatg	2760
	tataacaaaa	ataaacgctg	aaagcatcta	agcqtqaaqc	cccctcaag	atgagatttc	2820
	ccatttcttc	ggaaagtaag	atccctgaaa	gatgatcagg	tagataggtt	tggagtggaa	2880
	gtgtagggat	acatggageg	gacaaatact	aatcqatcqa	ggacttaacc	aa	2932
	3 3- 3 3	55 5 5	-				
45							
	<210> 54						
	<211> 2923						
	<212> DNA						
	<213> Stapl	ylococcus a	ureus				
50		-					
	<400> 54						
	gattaagtta	ttaagggcgc	acggtggatg	ccttggcact	agaagccgat	gaaggacgtt	60
						tttccgaatg	
	gggaaaccca	gcatgagtta	tgtcatgtta	tcgatatgtg	aatacatagc	atatcagaag	180
55	gcacacccgg	agaactgaaa	catcttagta	cccggaggaa	gagaaagaaa	attcgattcc	240
	cttagtagcg	gcgagcgaaa	cgggaagagc	ccaaaccaac	aagcttgctt	gttggggttg	300

WO 03/054162 PCT/US02/41014 28/52

		tetegggggt	tagaaagaag	gacattagac	gaatcatctg	gaaagatgaa	360
						atcctgagta	
						gctaaatact	
						cccggaaggg	
5						aatgggtgat	
-						aagcagtaaa	
						gtagacccga	
						gaggaccgaa	
						ccaatcgaac	
10						atgattattg	
						gacaaactcc	
						tgttcgaaag	
	ggaaacagcc	cagaccacca	gctaaggtcc	caaaatatat	gttaagtgga	aaaggatgtg	1080
	gcgttgccca	gacaactagg	atgttggctt	agaagcagcc	atcatttaaa	gagtgcgtaa	1140
15	tageteacta	gtcgagtgac	actgcgccga	aaatgtaccg	gggctaaaca	tattaccgaa	1200
	gctgtggatt	gtcctttgga	caatqqtaqq	agagcgttct	aagggcgttg	aagcatgatc	1260
	gtaaggacat	gtggagcgct	tagaagtgag	aatgccggtg	tgagtagcga	aagacgggtg	1320
						tctgggttag	
	tegggteeta	agctgaggcc	gacaggcgta	ggcgatggat	aacaggttga	tattcctgta	1440
20						cgtgcgattg	
	gattgcacgt	ctaagcagta	aggctgagta	ttaggcaaat	ccggtactcg	ttaaggctga	1560
	gctgtgatgg	ggagaagaca	ttgtgtcttc	gagtcgttga	tttcacactg	ccgagaaaag	1620
	cctctagata	gaaaataggt	gcccgtaccg	caaaccgaca	caggtagtca	agatgagaat	1680
						gtaacttcgg	
25	gagaaggggt	gctctttagg	gttaacgccc	agaagagccg	cagtgaatag	gcccaagcga	1800
	ctgtttatca	aaaacacagg	tctctgctaa	accgtaaggt	gatgtatagg	ggctgacgcc	1860
						aatcgaagcc	
	ccagtaaacg	gcggccgtaa	ctataacggt	cctaaggtag	cgaaattcct	tgtcgggtaa	1980
••	gttccgaccc	gcacgaaagg	cgtaacgatt	tgggcactgt	ctcaacgaga	gactcggtga	2040
30	aatcatagta	cctgtgaaga	tgcaggttac	ccgcgacagg	acggaaagac	cccgtggagc	2100
						ggagcctttg	
						tgtgttggct	
						ttgactgggg	
26						gttggaaatc	
35						cgagcagggt	
						caacggataa	
						ggggaggttt ggttgggctg	
40	atageteate	aaagcggcac	atttgagaga	agetateett	agtagagagag	ttcggtccct gaccgggatg	2700
70	gacatacete	taatataaa	attateatae	caacaacata	agtacgagag	tatgtgtgga	2760
	gacatacccc	cctcasacca	tctaaccate	aaaccccct	caacatcaca	tttcccaact	2820
	tegggataagt	getgaaagea	agatgatgag	attaataaat	tragargaga	agcatggtga	2880
				aagacttaat		~	2923
45	cacgeggage	caucaucus	canoognors				
	<210> 55						
	<211> 2900						
	<212> DNA						
50	<213> Stre	tococcus m	itans				
	<400> 55						
	gttaagttaa	taagggcgca	cggtggatgc	ctaggcacta	ggagccgatg	aaggacgtga	60
	cgaacgacga	catgctttgg	ggagctgtaa	gtaagccttg	atccagagat	atccgaatgg	120
55	gggaacccaa	caggtaatgc	ctgttatcca	taactgttaa	ggttatgaga	aggaagacgc	180
	agtgaactga	aacatctcag	tagctgcagg	aagagaaagc	aagagcgatt	gcctcagtag	240

```
cggcgagcga agaggcagga gggcaaacca gagtgtttac actctggggt tgtaggactg 300
    cgataaagca gccaagggaa tagaagaaga ctctgggaag agtcgccaga gagagtaaga 360
    gcctcgtatt tgaaattcac ttgatgccaa gcaggatcct gagtacggcg ggacacgagg 420
    aatcccgtcg gaatctggga ggcccatctc ccaaccctaa atactcccta gtgaccgata 480
    gtgaaccagt accgtgagg aaaggtgaaa agtaccccgg aaggggagtg aaagagaacc 540
    tgaaaccgtg tgcttacaag aagttcgagc ccgttaatgg gtgagagcgt gccttttgta 600
    qaatqaaccq qcqaqttacq tttacqtqcq aqqttaaqtt qaaqaqacqq aqccgtaggq 660
    aaaccqaqtc tqaaaaqqqc qqttaaqtac qtagatgtag acccgaaacc aagtgaccta 720
    cccatgagca ggttgaaggt gcggtaaaac gcactggagg accgaaccag gacacgttga 780
10
    aaagtgtttg gatgacttgt gggtagcgga gaaattccaa acgaacttgg agatagctgg 840
    ttctctccga aatagcttta gggctagcgt cggtcgcgag actcttggag gtagagcact 900
    gtttgattga ggggtccatc ccggattacc aatctcagat aaactccgaa tgccaacgag 960
    ttaagaccgg cagtcagact gcgagtgcta agatccgtag tcgaaaggga aacagcccag 1020
    accaccaget aaggteecca aataattgtt aagtggaaaa ggatgtgggg ttgcacagac 1080
    aactaggatg ttagcttaga agcagctatt cattcaaaga gtgcgtaata gctcactagt 1140
    cgagtgaccc tgcgccgaaa atgtaccggg gctgaaacaa tttaccgaag ctgtggatcc 1200
    cttaggggat ggtaggagag cgttctatgt gcgcagaagg tgtaccgcaa ggagcgctgg 1260
    aqtqcataqa aqtqaqaatq ccqqtatqaq taqcqtaaqa caqqtqaqaa tcctqtccac 1320
    cqtaagacta aggattccag gggaaggctc gtccgccctg ggttagtcgg gacctaagga 1380
20
    gagaccgata ggtgtatccg atgggcaaca ggttgatatt cctgtactag agtattgagt 1440
    gaaggaggga cgcagcaggc taactagagc gtgcgattgg aagagcacgt ccaagcagtg 1500
    aggtgaggac tgagtcaaat gcttagttct gcgccaccaa gctgtgacgg ggagcgaagt 1560
    ttagtagcga agctagtgat gtcactctgc caagaaaagc ttctagcgtt aatgaatact 1620
    ctacccgtac cgcaaaccga cacaggtagt cgaggcgagt agcctcaggt gatcgagcga 1680
    actotogtta aggaactogg caaaatggcc cogtaactto gggagaaggg gogotggcga 1740
    taagtcagcc gcagtgaaaa ggcccaagca actgtttatc aaaaacacag ctctctgcga 1800
    aatogtaaga tgaagtatag ggggtgacgc ctgcccggtg ctggaaggtt aagaggagcg 1860
    cttagacgtt tgtcgaaggt gtgaattgaa gccccagtaa acggcggccg taactataac 1920
    ggtcctaagg tagcgaaatt ccttgtcggg taagttccga cccgcacgaa aggcgtaatg 1980
30
    atttgggcac tgtctcaacg agagactcgg tgaaatttta gtacctgtga agatgcaggt 2040
    tacccgcgac aggacggaaa gaccccatgg agctttactg cagtttgata ttgcgtatct 2100
    gttacacatg tacaggatag gtaggagcca aggaagagtg aacgctagtt tacttggagg 2160
    cqttqttqqq atactaccct tqtqtqatqq ctactctaac ccqqtaqqtt gatcatctac 2220
    qqaqacaqtq tetgacgggc agtttgactg gggcggtcgc etectaaagc gtaacggagg 2280
35
    cgcccaaagg ttccctcaga ctggttggaa atcagtcgta gagtgtaaag gtataaggga 2340
    gcttgactgc gagacagaca agtcgagcag ggacgaaagt cgggcttagt gatccggtgg 2400
    taccgtatgg aagggccatc gctcaacgga taaaagctac cctggggata acaggcttat 2460
    ctccccaag agttcacatc gacggggagg tttggcacct cgatgtcggc tcgtcgcatc 2520
    ctggggctgt agtcggtccc aagggttggg ctgttcgccc attaaagcgg cacgcgagct 2580
40
    gggttcagaa cgtcgtgaga cagttcggtc cctatccgtc gcgggcgaag gaaatttgag 2640
    aqqatctgct cctagtacga gaggaccaga gtggacttac cgctggtgta ccagttgttc 2700
    tgccaaqagc atcgctgggt agctaagtag ggaggggata aacgctgaaa gcatctaagt 2760
    gtgaagccc cctcaagatg agatttccca taacgttcag ttagtaagag ccctgaaaga 2820
    agaacaggta gataggttgg gagtggaagc gttgtgagac gtgaagcgga ccaatactaa 2880
45
    tcgctcgagg acttatccaa
```

<210> 56 <211> 2902 <212> DNA

<213> Streptococcus pneumoniae

<400> 56 gyttaagtta ataagggcgc acggtggatg cettggcact aggagccgac gaaggacgtg 60 55 acaaacgacg atatgcettg ggtagetgta agtaagcgat gatccaggga tttccgaatg 120 ggggaaccca acaggtaata cctgttaccc acatcgtta aggagtgtag aggagaagcg 180

	cagtgaactg	aaacatctaa	gtagctgcag	qaaqaqaaaq	caaaagcgat	tqccttaqta	240
					ctcttcgggg		
					gatcagccaa		
	agectegtat	ttaaaatagt	ctttgtactt	agcagtatcc	tgagtacggc	gggacacgtg	420
5	aaatcccqtc	ggaatctggg	aggaccatct	cccaacccta	aatactccct	agtgaccgat	480
-					ggagggagt		
					ggtgagagcg		
					tgaagagacg		
					gacccgaaac		
10					gaccgaacca		
					aacgaacttg		
	atteteteca	aaatagcttt	aggetageg	tcgacattag	agattcttgg	aggtagagca	900
	cratttagat	gagggtcca	tcccggatta	ccaatctcag	ataaactccg	aatqccaatq	960
	aattatooto	garagtraga	ctacaaatac	taagatccgt	agtcgaaagg	gaaacageee	1020
15	agaccaccag	ctaaggtccc	aaaataatto	ttaagtggaa	aaggatgtgg	ggttgcacag	1080
	acaactagga	tottagetta	gaagcagcta	ttcattcaaa	gagtgcgtaa	tageteacta	1140
	atcaagtgac	cctgcgccga	aaatgtaccg	gggctaaaac	aatttaccga	agctgtggat	1200
					aaggtatacc		
	ctogaacgca	tagaagtgag	aatgccggta	tgagtagcga	aagacaggtg	agaatcctgt	1320
20	ccaccataaa	actaaggttt	ccaggggaag	acteateeac	cctgggttag	tcgggaccta	1380
					tattcctgta		
					ttggaagagt		
					ttgagctgtg		
					aagcttctag		
25	tactctaccc	gtaccgcaaa	ccqacacaqq	tagtcgaggc	gagtagcctc	aggtgagcga	1680
	gagaactctc	gttaaggaac	tcqqcaaaat	gaccccgtaa	cttcgggaga	aggggtgctg	1740
	acttaaagtc	agccgcagtg	aataggccca	agcaactgtt	tatcaaaaac	acagetetet	1800
	gctaaatcqt	aaqatqatqt	atagggggtg	acqcctgccc	ggtgctggaa	ggttaagagg	1860
	agtgcttagc	qtaaqcqaaq	gtatgaattg	aagccccagt	aaacggcggc	cgtaactata	1920
30	accotcctaa	ggtagcgaaa	tteettatea	qqtaaqttcc	gacccgcacg	aaaggcgtaa	1980
					tagtacctgt		
	attacccqcq	acaggacgga	aaqaccccat	ggagetttae	tgcagtttga	tattgagtgt	2100
	ctqtaccaca	tqtacaqqat	aggtaggagt	ctaagagatc	gggacgccag	tttcgaagga	2160
	gacgetgttg	qqatactacc	cttgtgttat	ggccactcta	acccagatag	gtgatcccta	2220
35	tcqqaqacag	tgtctgacgg	gcagtttgac	tggggcggtc	gcctcctaaa	aggtaacgga	2280
	ggcgcccaaa	ggttccctca	gaatggttgg	aaatcattcg	cagagtgtaa	aggtataagg	2340
	gagettgact	gcgagagcta	caactcgagc	agggacgaaa	gtcgggctta	gtgatccggt	2400
	ggttccgtat	ggaagggcca	tcgctcaacg	gataaaagct	accctgggga	taacaggctt	2460
	atctccccca	agagttcaca	tcgacgggga	ggtttggcac	ctcgatgtcg	gctcgtcgca	2520
40	teetgggget	gtagtcggtc	ccaagggttg	ggctgttcgc	ccattaaagc	ggcacgcgag	2580
	ctgggttcag	aacgtcgtga	gacagttcgg	tccctatccg	tcgcgggcgt	aggaaatttg	2640
	agaggatctg	ctcctagtac	gagaggacca	gagtggactt	accgctggtg	taccagttgt	2700
	cttgccaaag	gcatcgctgg	gtagctatgt	agggaaggga	taaacgctga	aagcatctaa	2760
	gtgtgaaacc	cacctcaaga	tgagatttcc	catgattata	tatcagtaag	agccctgaga	2820
45	gatgatcagg	tagataggtt	agaagtggaa	gtgtggcgac	acatgtagcg	gactaatact	2880
	aatagctcga	ggacttatcc	aa	'			2902
	_						

<210> 57 50 <211> 2901

<212> DNA

<213> Streptococcus pyogenes

<400> 57

ggttaagtta ataagggcgc acggtggatg cettggcact agaagcegaa gaaggacgtg 60 actaacgacg aaatgetttg gggagetgta agtaagcgct gatecagaga tgtecgaatg 120

```
qqqqaacccg gcatgtaatg catgtcatcc atgactgtta aggtcatgag aaggaagacg 180
    caqtgaactg aaacatctaa gtagctgcag gaagagaaag caaacgcgat tgccttagta 240
    gcggcgagcg aaacggcagg agggcaaacc gaggagttta ctcctcgggg ttgtaggact 300
    gcgaagtggg acataaagtt aatagaagaa ttacctggga aggtaagcca aagagagtaa 360
    cagcetegta tttaaaattg actttageee tagcaqtate etgagtacgg egagacacge 420
    gaaatctcgt cggaatctgg gaggaccatc tcccaaccct aaatactctc tagtgaccga 480
    tagtgaacca qtaccqtqaq qqaaaqqtga aaaqcacccc qqqaqqqqaq tqaaatagaa 540
    cctgaaaccg tgtgcctaca acaagttcga gcccgttaat gggtgagagc gtgccttttg 600
    taqaatqaac cqqcgaqtta cgatatgatg cgaggttaag ttgaagagac ggagccgtag 660
10
    qqaaaccgaq tettaatagg gegteatagt atcatgttgt agaccegaaa ecatgtgace 720
    tacccatgag caggttgaag gtgtggtaaa acgcactgga ggaccgaacc agggcacgtt 780
    gaaaagtgct tggatgactt gtgggtagcg gagaaattcc aaacgaactt ggagatagct 840
    ggttetetee gaaatagett tagggetage gtegatgtta agtetettgg aggtagagea 900
    ctqtttqqqt qaqqqtcca tcccqqatta ccaatctcaq ataaactccg aatgccaacg 960
    agatataatc ggcagtcaga ctgcgagtgc taagatccgt agtcgaaagg gaaacagccc 1020
    aqaccaccaq ctaaggtccc aaaataactg ttaagtggaa aaggatgtgg ggttgcacag 1080
    acaactagga tgttagctta gaagcagcta ttcattcaaa gagtgcgtaa tagctcacta 1140
    gtcgagtgac cctgcgccga aaatgtaccg gggctaaaac agtttaccga agctgtggat 1200
    gacacaaaag tgtcatggta ggagagcgtt ctatgtgtga agaaggtgta ccgtgaggag 1260
20
    cgctggaacg catagaagtg agaatgccgg tatgagtagc gaaagacagg tgagaatcct 1320
    gtccaccqta aqactaaqqt ttccagggga aggctcgtcc gccctgggtt agtcgggacc 1380
    taaqqaqaqa ccqaaaqqtg tatccgatgg ccaacaggtt gatattcctg tactagagta 1440
    tataqtqatq qaqqqacqca gtaggctaac taaaccggac gattggaaga gtccggctaa 1500
    qcaqtqaqqt qtaagatqag tcaaatgctt atctttataa cattgagctg tgatggggag 1560
    cqaattttag tagcgaagtt agtgatgtca cactgccaag aaaagcttct agcgtttaat 1620
    gatactctac ccgtaccgca aaccgacaca ggtagtcgag gcgagtagcc tcaggtgatc 1680
    gagagaacto togttaagga actoggcaaa atgaccoogt aacttoggga gaaggggtgc 1740
    tgacttaggt cagccgcagt gaataggccc aagcaactgt ttatcaaaaa cacagctctc 1800
    tgctaaatcg taagatgatg tatagggggt gacgcctgcc cggtgctgga aggttaagag 1860
    gagggtttag cgcaagcgaa gatctgaatt gaagccccag taaacggcgg ccgtaactat 1920
    aacggtccta aggtagcgaa attccttgtc gggtaagttc cgacccgcac gaaaggcgta 1980
    atgatttqqq cactgtctca acgagagact cggtgaaatt ttagtacctg tgaagatgca 2040
    ggttacccgc gacaggacgg aaagacccca tggagcttta ctgcagtttg atattgagta 2100
    tctgtaccac atgtacagga taggtaggag ccattgactt cgggacgcca gtttcgaatg 2160
35
    aggogttgtt gggatactac cottgtgtta tggctactct aacccagata ggttatccct 2220
    atcggagaca gtgtctgacg ggcagtttga ctggggcggt cgcctcctaa agagtaacgg 2280
    aggogoccaa aggttocoto agattggttg gaaatcaato goagagtgta aaggtataag 2340
    qqaqcttqac tgcqaqaqct acaactcqaq caggqacqaa agtcgggctt agtgatccgg 2400
    tggtaccgaa tggaagggcc atcgctcaac ggataaaagc taccctgggg ataacaggct 2460
    tatctccccc aagagttcac atcgacgggg aggtttggca cctcgatgtc ggctcgtcgc 2520
    atcctggggc tgtagtcggt cccaagggtt gggctgttcg cccattaaag cggcacgcga 2580
    gctgggttca gaacgtcgtg agacagttcg gtccctatcc gtcgcgggcg taggaaattt 2640
    gagaggatet geteetagta egagaggace agagtggact tacegetggt gtaceagttg 2700
    tettgecaaa ggeategetg ggtagetatg tagggaaggg ataagegetg aaageateta 2760
45
    agtgcgaagc ccccctcaag atgagatttc ccatgatttt atatcagtaa gagccctgag 2820
    agatgatcag gtagataggt taggagtgta agtgtagcga tacatgtagc ggactaatac 2880
    taatagctcg aggacttatc c
                                                                      2901
```

^{50 &}lt;210> 58 <211> 3107 <212> DNA <213> Mycobacterium avium

^{55 &}lt;400> 58 tqtqtqtaag taagtqttta agggcgcatg gtggatgcct tggcatcgag agccgatgaa 60

	ggacgtggga	ggctgcgata	tgcctcgggg	agctgtcaac	cgagcattga	tccgaggatt	120
	tccgaatggg	ggaacccagc	acgagtgatg	tcgtgttacc	cgtatctgaa	tatatagggt	180
	gcgggaggta	acgcggggaa	gtgaaacatc	tcagtacccg	taggagaaga	aaacaattgt	240
	gattccgtca	gtagtggcga	gcgaaccgga	acaggctaaa	ccgcatgcat	ggacaaccgg	300
5	gtaggggttg	tgtgtgcggg	gttgtgggat	tgatatgtct	cagctctacc	tggctgaggg	360
	gtagtcagaa	agtgtcgtgg	ttagcggaag	tggcctggga	cggcccgccg	tagacggtga	420
	qaqcccqqta	cgcgaaaacc	cggcacctgc	cttatatcaa	cacccgagta	gcagcgggcc	480
	cgtggaatct	gctgtgaatc	tgccgggacc	acccggtaag	cctaaatact	tctcgatgac	540
	cgatagcgga	ttagtaccgt	gagggaatgg	tgaaaagtac	cccgggaggg	agtgaaatag	600
10	tacctgaaac	cgtgtgccta	caatccgtca	gagcctcctc	gtggggtgat	ggcgtgcctt	660
	ttgaagaatg	agcctgcgag	tcagggacac	gtcgcgaggt	taacccgtgc	ggggtagccg	720
	cagcgaaagc	gagtctgaat	agggcgcatc	ccctttgggg	tgtagtggcg	tgttctggac	780
	ccgaagcgga	gtgatctacc	catggccagg	gtgaagcgcg	ggtaagaccg	cgtggaggcc	840
	cgaacccact	taggttgaag	actgagggga	tgagctgtgg	gtaggggtga	aaggccaatc	900
15	aaactccgtg	atagctggtt	ctccccgaaa	tgcatttagg	tgcagcgttg	cgtggttcac	960
	cacggaggta	gagctactgg	atggccgatg	ggccctacta	ggttactgac	gtcagccaaa	1020
	ctccgaatgc	cgtggtgtaa	aagcgtggca	gtgagacggc	gggggataag	ctccgtacgt	1080
	cgaaagggaa	acagcccaga	tcgccggcta	aggcccctaa	gcgtgtgcta	agtggaaaag	1140
	gatgtgtagt	cgcagagaca	accaggaggt	tggcttagaa	gcagccatcc	ttgaaagagt	1200
20	gcgtaatagc	tcactggtca	agtgattatg	cgccgataat	gtagcggggc	tcaagcacac	1260
	cgccgaagcc	gcggcacatt	catctttacg	gtggatgtgg	gtaggggagc	gtcccccatt	1320
	cagcgaagct	ccgggtgacc	ggtggtggag	ggtgggggag	tgagaatgca	ggcatgagta	1380
	gcgataaggc	aagtgagaac	cttgcccgcc	gtaagaccaa	gggttcctgg	gccaggccag	1440
	tccgcccagg	gtgagtcggg	acctaaggcg	aggccgacag	ggtagtcgat	ggacaacggg	1500
25	ttgatattcc	cgtacccgtg	tatgggcgtc	cctgatgaat	cagcggtact	aaccacccaa	1560
	aaccggatcg	accattcccc	ttcgggggcg	tggcgattcg	gggctgcgtg	ggaccttcgc	1620
	tggtagtagt	caagcaatgg	ggtgacgcag	gaaggcagcc	gtaccagtca	gtggtaatac	1680
	tggggcaagc	ccgtagagag	cgataggcaa	atccgtcgct	cactaatcct	gagaggtgat	1740
	gcatagccgg	ttgaggcgaa	ttcggtgatc	ctctgctgcc	aagaaaagcc	tctagcgagc	1800
30	acatacacgg	cccgtacccc	aaaccaacac	aggtggtcag	gtagagaata	ccaaggcgta	1860
	cgagataact	atggttaagg	aactcggcaa	aatgcccccg	taacttcggg	agaagggggc	1920
	ccggaatacc	gtgaacaccc	ttgcggtggg	agcgggattc	ggccgcagaa	accagtgggt	1980
	agcgactgtt	tactaaaaac	acaggtccgt	gcgaagtcgc	aagacgatgt	atacggactg	2040
	acgcctgccc	ggtgctggaa	ggttaagagg	acccgttaac	ccgtaagggt	gaagcggaga	2100
35	atttaagccc	cagtaaacgg	cggtggtaac	tataaccatc	ctaaggtagc	gaaattcctt	2160
	gtcgggtaag	ttccgacctg	cacgaatggc	gtaacgactt	cccaactgtc	tcaaccatag	2220
	actcggcgaa	attgcactac	gagtaaagat	gctcgttacg	cgcggcagga	cgaaaagacc	2280
	ccgggacctt	cactacaact	tggtattggt	gttcggtacg	gtttgtgtag	gataggtggg	2340
	agactttgaa	gcacagacgc	cagtttgtgt	ggagtcgttg	ttgaaatacc	actctgatcg	2400
40	tattggacac	ctaacgtcga	accettateg	ggttcacgga	cagtgcctgg	cgggtagttt	2460
	aactggggcg	gttgcctcct	aaaatgtaac	ggaggcgccc	aaaggttccc	tcaacctgga	2520
	cggcaatcag	gtggcgagtg	taagtgcaca	agggagcttg	actgcgagac	ttacaagtca	2580
	agcagggacg	aaagtcggga	ctagtgatcc	ggcacccccg	agtggaaggg	gtgtcactca	2640
	acggataaaa	ggtaccccgg	ggataacggg	ctgatcttcc	ccaagagtcc	atatcgacgg	2700
45	gatggtttgg	cacctcgatg	teggetegte	gcatcctggg	gctggagcag	gtcccaaagg	2760
	ttgggctgtt	cgcccattaa	agcggcacgc	gagctgggtt	tagaacgtcg	tgagacagtt	2820
	cggtctctat	ccgccgcgcg	cgtcagaaac	ttgaggaaac	ctgtccctag	tacgagagga	2880
	ccgggacgga	cgaacctctg	gtataccagt	tgtcccacca	ggggcacggc	tggatagcca	2940
	cgttcggaca	ggataaccgc	tgaaagcatc	taagcgggaa	accttctcca	agatcaggtt	3000
50					ggattgatag	gccagacctg	3060
	gaagctcagt	aatgagtgca	gggaactggc	actaactggc	cgaaagc		3107

55

WO 03/054162 PCT/US02/41014

<213> Mycobacterium tuberculosis

<400> 59

	<400> 59						
	ttgtaagtgt	ctaagggcgc	atggtggatg	ccttggcatc	gagagccgat	gaaggacgtg	60
5	ggaggetgeg	atatgcctcg	gggagctgtc	aaccgagcgt	ggatccgagg	atttccgaat	120
	qqqqaaaccc	agcacgagtg	atgtcgtgct	acccqcatct	gaatatatag	gqtqcqqqaq	180
			atctcagtac				
			cggaacaggc				
			ggaggatatg				
10			aagtggcctg				
			tgcctagtat				
			accacccggt				
			tggtgaaaag				
			gtcagagcct				
15			gcctgcgagt				
			agtctgaata				
			ccgaagcgga				
			cgaacccact				
			aaactccgtg				
20			cgcggaggta				
			ctccgaatgc				
			gaaagggaaa				
			atgtgcagtc				
			cgtaatagct				
25			gccgaagccg				
			gcgaagccac				
			cgacaaggca				
			ccgcccaggg				
			ttgatattcc				
30			aaccggatcg				
-			tggtagtagt				
			tggggcaagc				
			acgcatagcc				
			gcacacacac				
35			tacgagataa				
33			gaccggaata				
			ggagcgactg				
			tgacgcctgc				
			gaatttaagc				
40			ttgtcgggta				
			agactcggcg				
			ccccgggacc				
			ggagactgtg				
			cgtattgggc				
45			tttaactggg				
45			ggacggcaat				
			tcaagcaggg				
			tcaacggata				
			cgggatggtt				
50			gggttgggct				
50							
			gtteggtete				
			ggaccgggac ccacgttcgg				
55			gtttctcacc				
55			ctggaagctc	agcaatgggt	grayygaact	ggcgccaacc	3138
	ggccgaaaac	LLACAACA					2120

<210> 60

WO 03/054162 PCT/US02/41014

```
<211> 2903
    <212> DNA
    <213> Escherichia coli
    <400> 60
    ggttaagega etaagegtae aeggtggatg ceetggeagt cagaggegat gaaggaegtg 60
10
    ctaatctgcg ataagcgtcg gtaaggtgat atgaaccgtt ataaccggcg atttccgaat 120
    ggggaaaccc agtgtgattc gtcacactat cattaactga atccataggt taatgaggcg 180
    aaccggggga actgaaacat ctaagtaccc cgaggaaaag aaatcaaccg agattccccc 240
    agtageggeg agegaacggg gaggagecca gageetgaat cagtgtgtgt gttagtggaa 300
    gcqtctggaa aggcgcgcga tacagggtga cagccccgta cacaaaaatg cacatactgt 360
15
    gagetegatg agtagggegg gacaegtggt atcetgtetg aatatggggg gaccateete 420
    caaggetaaa tacteetgae tgacegatag tgaaccagta cegtgaggga aaggegaaaa 480
    gaaccccggc gaggggagtg aaaaagaacc tgaaaccgtg tacgtacaag cagtgggagc 540
    ctcttttatg gggtgactgc gtaccttttg tataatgggt cagcgactta tattctgtag 600
    caaggttaac cgaatagggg agccgaaggg aaaccgagtc ttaaccgggc gttaagttgc 660
20
    agggtataga cccgaaaccc ggtgatctag ccatgggcag gttgaaggtt gggtaacact 720
    aactggagga ccgaaccgac taatgttgaa aaattagcgg atgacttgtg gctgggggtg 780
    aaaggccaat caaaccggga gatagctggt tctccccgaa agctatttag gtagcgcctc 840
    gtgaattcat ctccgggggt agagcactgt ttcggcaagg gggtcatccc gacttaccaa 900
    cccgatgcaa actgcgaata ccggagaatg ttatcacggg agacatacgg cgggtgctaa 960
25
    cgtccgtcgt gaagagggaa acaacccaga ccgccagcta aggtcccaaa gtcatggtta 1020
    aqtqqqaaac gatqtqqqaa gqcccaqaca gccaqqatqt tqqcttaqaa gcaqccatca 1080
    tttaaagaaa gcgtaatagc tcactggtcg agtcggcctg cgcggaagat gtaacggggc 1140
    taaaccatgc accgaagctg cggcagcgac actgtgtgtt gttgggtagg ggagcgttct 1200
    gtaagcctgt gaaggtgtac tgtgaggtat gctggaggta tcagaagtgc gaatgctgac 1260
    ataagtaacg ataaagcggg tgaaaagccc gctcgccgga agaccaaggg ttcctgtcca 1320
    acgttaatcg gggcagggtg agtcgacccc taaggcgagg ccgaaaggcg tagtcgatgg 1380
    qaaacaqqtt aatattcctg tacttggtgt tactgcgaag gggggacgga gaaggctatg 1440
    ttqqccgggc gacggttgtc ccggtttaag cgtgtaggct ggttttccag gcaaatccgg 1500
    aaaatcaagg ctgaggcgtg atgacgaggc actacggtgc tgaagcaaca aatgccctgc 1560
35
    ttccaggaaa agcctctaag catcaggtaa catcaaatcg taccccaaac cgacacaggt 1620
    ggtcaggtag agaataccaa ggcgcttgag agaactcggg tgaaggaact aggcaaaatg 1680
    gtgccgtaac ttcgggagaa ggcacgctga tatgtaggtg aagtccctcg cggatggagc 1740
    tgaaatcagt cgaagatacc agctggctgc aactgtttat taaaaacaca gcactgtgca 1800
    aacacgaaag tggacgtata cggtgtgacg cctgcccggt gccggaaggt taattgatgg 1860
40
    ggtcagcgca agcgaagctc ttgatcgaag ccccggtaaa cggcggccgt aactataacg 1920
    gtcctaaggt agcgaaattc cttgtcgggt aagttccgac ctgcacgaat ggcgtaatga 1980
    tggccaggct gtctccaccc gagactcagt gaaattgaac tcgctgtgaa gatgcagtgt 2040
    accogggga agacggaaag accocgtgaa cotttactat agottgacac tgaacattga 2100
    qccttqatqt qtaqqataqq tqqqaqqctt tgaaqtqtqq acqccaqtct qcatqqaqcc 2160
45
    gaccttgaaa taccaccctt taatgtttga tgttctaacg tggacccgtg atccgggttg 2220
    cggacagtgt ctggtgggta gtttgactgg ggcggtctcc tcctaaagag taacggagga 2280
    qcacqaaqqt tqqctaatcc tqqtcgqaca tcaggaggtt agtgcaatgg cataagccag 2340
    cttqactqcq aqcqtqacqq cqcqaqcaqq tqcqaaaqca qqtcataqtq atccqqtqqt 2400
    tctqaatqqa agggccatcg ctcaacggat aaaaggtact ccggggataa caggctgata 2460
    ccgcccaaga gttcatatcg acggcggtgt ttggcacctc gatgtcggct catcacatcc 2520
50
    tggggctgaa gtaggtccca agggtatggc tgttcgccat ttaaagtggt acgcgagctg 2580
    ggtttagaac gtcgtgagac agttcggtcc ctatctgccg tgggcgctgg agaactgagg 2640
    ggggctgctc ctagtacgag aggaccggag tggacgcatc actggtgttc gggttgtcat 2700
    gccaatggca ctgcccggta gctaaatgcg gaagagataa gtgctgaaag catctaagca 2760
55
    cqaaacttqc cccqaqatqa gttctccctg accctttaag ggtcctgaag gaacgttgaa 2820
    gacqacqacq ttgataggcc gggtgtgtaa gcgcagcgat gcgttgagct aaccggtact 2880
```

45

50

55

aatqaaccqt qaqqcttaac ctt

2903

<210> 61 <211> 2903 <212> DNA <213> Klebsiella pneumoniae <400> 61 ggttaagcga ctaagcgtac acggtggatg ccctggcagt cagaggcgat gaaggacgtg 60 ctaatctqcq aaaagcgtcg gtaaggtgat atgaaccgtt ataaccggcg atgtccgaat 120 ggggaaaccc agtgcaattc gttgcactat cgttaactga atacataggt taacgaggcg 180 aaccggggga actgaaacat ctaagtaccc cgaggaaaag aaatcaaccg agattccccc 240 agtageggeg agegaaeggg gageageeca gagtetgaat eagettgtgt gttagtggaa 300 cggtctggaa agtccgacgg tacagggtga tagtcccgta caccaaaatg cacaggctgt 360 gaactegaag agtagggegg gacacgtggt atcetgtetg aatatggggg gaccateete 420 caaggetaaa tacteetgae tgaccgatag tgaaccagta cegtgaggga aaggegaaaa 480 gaaccccggc gaggggagtg aaaaagaacc tgaaaccgtg tacgtacaag cagtgggagc 540 accttegggt gtgactgegt accttttgta taatgggtca gegaettata ttetgtagea 600 aggttaaccg tataggggag ccgcagggaa accgagtctt aactgggcgt taagttgcag 660 ggtatagacc cgaaacccgg tgatctagcc atgggcaggt tgaaggttgg gtaacactaa 720 ctggaggacc gaaccgacta atgttgaaaa attagcggat gacttgtggc tgggggtgaa 780 aggccaatca aaccgggaga tagctggttc tccccgaaag ctatttaggt agcgcctcgt 840 gaactcatct tcgggggtag agcactgttt cggctagggg gtcatcccga cttaccaacc 900 cgatgcaaac tacgaatacc gaagaatgtt atcacgggag acacacggcg ggtgctaacg 960 25 tccgtcgtga agagggaaac aacccagacc gccagctaag gtcccaaagt catggttaag 1020 tgggaaacga tgtgggaagg cacagacagc caggatgttg gcttagaagc agccatcatt 1080 taaagaaagc gtaatagctc actggtcgag tcggcctgcg cggaagatgt aacggggcta 1140 aaccatgcac cgaagctgcg gcagcgacac tatgtgttgt tgggtagggg agcgttctgt 1200 aageetgega aggtgtgetg tgaggeatge tggaggtate agaagtgega atgetgacat 1260 aagtaacgat aaagcgggtg aaaagcccgc tcgccggaag accaagggtt cctgtccaac 1320 gttaatcggg gcagggtgag tcgaccccta aggcgaggcc gaaaggcgta gtcgatggga 1380 aacaggttaa tattootgta ottggtgtta otgogaaggg gggacggaga aggotatgtt 1440 agccgggcga cggttgtccc ggtttaagca tgtaggctgg ttgtccaggc aaatccggat 1500 aatcaagget gaggtgtgat gacgaggeac tacggtgetg aagtaacaaa tgetetgett 1560 35 ccaggaaaag cctctaagca tcaggtaaca tcaaatcgta ccccaaaccg acacaggtgg 1620 tcaggtagag aataccaagg cgcttgagat aactcgggtg aaggaactag gcaaaatggt 1680 gccgtaactt cgggagaagg cacgctggtg tgtaggtgaa gcccctgccg ggtggagctg 1740 agaccagtcg aagataccag ctggctgcaa ctgtttatta aaaacacagc actgtgcaaa 1800 40 cacqaaagtg gacgtatacg gtgtgacgcc tgcccggtgc cggaaggtta attgatgggg 1860

ttaicogiaa gagaaagoto tigaitogaag ocooggiaaa oggoggoogt aactataacg 1920 gicotaaggi agcgaaatto ottutogggi aagitooga otgocaggat ggogiaatga 1980 tggocaggot gotocaoco gagaatoagi gaaattgaac togocytysaa gatyaatgi 2040 accoogg

aacettgaaa kaccacectt taatgittga töttetaacg tiggececte aceggggttg 2220 eggacagtgt etggytggta gittgactgg ggeggtetet teceaaageg taaceggagga 2280 gacagaaggt tagetaatee tggteggaca teaggaggit agigeaatgg cataagetag 2340 ettgactgcg agegtgacgg egeagcagg tgegaaaga gjteataatg aceggggtag 240 tetgaatga agggecateg etcaacggat aaaaggtact coggggataa aggetgata 2460

cogoccaaga gitcatatog acggoggigt tiggoacoto gatgtoggot catcacatoc 2520 tggggottgaa giaggicoca agggitatggo tgitogocat itaaagiggi acgcgagetg 2580 ggittagaac gicgigagac agitoggico cialotogog igggogotgg agaaitgagg 2640 ggggotgoto ciagiacgag aggacoggag iggacogota aciggigito gggitgicat 2700

gccaatggca ctgcccggta gctaaatgcg gaagagataa gtgctgaaag catctaagca 2760 cqaaacttgc cccgagatga gttctccctg agactttaag tctcctgaag gaacgttgaa 2820 36/52

qacqacqacq ttgataggcc gggtgtgtaa gcgcagcgat gcgttgagct aaccggtact 2880 aatgaaccgt gaggcttaac ctt <210> 62 <211> 2897 <212> DNA <213> Haemophilus influenzae 10 <400> 62 gtatagttaa gtgactaagc gtacaaggtg gatgccttgg caatcagagg cgaagaagga 60 cgtgctaatc tgcgaaaagc ttggatgagt cgataagagg cgtttaatcc aagatatccg 120 aatggggaaa cccagtagat gaagaatcta ctatcaacaa gtgaattcat agcttgttga 180 ggcaaaccgg gagaactgaa acatctaagt accccgagga aaagaaatca accgagattt 240 15 cgtcagtagc ggcgagcgaa agcgaaagag ccagtaagtg atagcaatat agtgaggaga 300 atqtqttqqq aagcacaatc aaagagggtg ataatcccgt atctaaaaac catattgtgg 360 tactaagcta acgagaagta gggcgggaca cgtgatatcc tgtttgaaga aggggggccc 420 atcctccaag gctaaatact cctgattgac cgatagtgaa ccagtactgt gaaggaaagg 480 cgaaaagaac cccggtgagg ggagtgaaat agaacctgaa accttgtacg tacaagcagt 540 ggagggagg gcaaccttqt qactqcgtac cttttgtata atgggtcagc gacttatatt 600 ttgtagcgag gttaaccgaa taggggagcc gaagggaaac cgagtcttaa ctgggcgaat 660 agttqcaaqq tataqacccq aaacccggtg atctagccat gggcaggttq aaqqttgggt 720 aacactaact ggaggaccga accgactaat gttgaaaaat tagcggatga cttgtggctg 780 qqqqtgaaag gccaatcaaa ccgggagata gctggttctc cccgaaatct atttaggtag 840 agcettgagg tgacacettt gggggtagag cactgttteg getaggggge catecegget 900 taccaacccg atgcaaacta cgaataccaa agagtgatac tcaggagaca cacggcgggt 960 gctaacgtcc gtcgtggaga gggaaacaac ccagaccgcc agctaaggtc cccaagtcta 1020 tattaagtgg gaaacgaagt gggaaggctt agacagctag gatgttggct tagaagcagc 1080 30 qqqqctqaaa tataqcaccq aagctgcggc atcagaattt attctgttgg gtaggggagc 1200 qttqtqtaag cggaagaagg ttcatcgaga ggtgggctgg acgtatcaca agtgcgaatg 1260 ctgacataag taacgataaa acgggtgaaa aacccgttcg ccggaagacc aagggttcct 1320 gtccaacgtt aatcggggca gggtgagtcg gctcctaagg cgaggctgaa aagcgtagtc 1380 gatgggaaac aggttaatat tootgtactt ggtaaagctg cgatgtgggg acggagtagg 1440 35 ttaggttatc gcactgttgg atatgtgcgt ttaagttggt aggtgggaag tttaggcaaa 1500 tecogactte ettaacacag agagatgatg acgaggetet acggagetga agtaactgat 1560 accacactte caggaaaage cactaagega aaggetttae taaacegtae tgaaaacega 1620 cacaggtggt caggtagaga atactcaggc gcttgagaga actcgggtga aggaactagg 1680 caaaatagca ccgtaacttc gggagaaggt gcgccggcgt agattgtaag ggctagcccc 1740 tgaaggttga accggtcgaa gataccagct ggctgcaact gtttattaaa aacacagcac 1800 tctgcaaaca cgaaagtgga cgtatagggt gtgatgcctg cccggtgctg gaaggttaat 1860 tgatggtgtc atcgaaagag aagcacctga tcgaagcccc agtaaacggc ggccgtaact 1920 ataacggtcc taaggtagcg aaatteettg tegggtaagt teegacetge acgaatggca 1980 taatqatqqc caqqctqtct ccacccqaqa ctcagtgaaa ttgaaatcgc cgtgaagatg 2040 cqqtqtaccc qcqqctagac ggaaagaccc cqtgaacctt tactatagct tgacactgaa 2100 cattqaattt tgatgtqtag gataggtqqq agcctttgaa gcagtgacgc cagtcattgt 2160 qqaqqqacc ttqaaatacc accetttaac gtttgatgtt ctaacgaaga tgacgaaacg 2220 tgqtctcgga cagtgtctgg tgggtagttt gactggggcg gtctcctccc aaagcgtaac 2280 ggaggagcac gaaggtttgc taatcacggt cggacatcgt gaggttagtg caatggtata 2340 agcaagctta actgcgagac agacaagtcg agcaggtacg aaagtaggtc atagtgatcc 2400 ggtggttctg aatggaaggg ccatcgctca acggataaaa ggtactccgg ggataacagg 2460 ctgataccgc ccaagagttc atatcgacgg cggtgtttgg cacctcgatg tcggctcatc 2520 acatectggg getgaagtag gteccaaggg tatggetgtt egecatttaa agtggtaege 2580 gagctgggtt tagaacgtcg tgagacagtt cggtccctat ctgccgtggg cgtaggatga 2640 ttgattgggg ctgctcctag tacgagagga ccggagtgga cgcatcactg gtgttccggt 2700 tgtgtcgcca gacgcattgc cgggtagcta aatgcggaag agataagtgc tgaaagcatc 2760

```
taagcacqaa acttgccaag agatgagtca tccctgactt taagtcagta agggttgttg 2820
    tagactacga cgtagatagg tigggtgtgt aagtgatgtg agtcattgag ctaaccaata 2880
    ctaattgccc gagaggc
5
    <210> 63
    <211> 2865
    <212> DNA
    <213> Bordetella bronchiseptica
10
    <220>
    <221> modified base
     <222> (622)
     <223> N = A, C, G or T/U
15
    gatcaaqcga ctaagtgcat atggtggatg ccttggcgat cacaggcgga tgaaggacgt 60
    agtagcctgc gaaaagctgc ggggagctgg caaacaagca ttgatccgca gatatccgaa 120
    togogaaacc cacogcaagc gotateceto getgaataca taggecagto gaggegaace 180
    gggtgaactg aaacatctca gtagctcgag gaaaagaaat caaccgagat tccgaaagta 240
    gtggcgageg aaatcggaag agcetttacg atttagcatt ttgcatagte gaacggaatg 300
    qaaaqtccqq ccqtaqcaqg tgatagccct gtagacgaat gcagagtgtg gaactaggcg 360
    taaqaqaagt agggcgggac acgtgaaatc ctgtctgaag atggggggac catcctccaa 420
    ggctaaatac tcgtgatcga ccgatagtga accagtaccg tgaggaaagg cgaaaagaac 480
25
    cccggaagga gtgaaataga tcctgaaacc gtatgcatac aacagtcgga gcctctttat 540
    ggggtgacgg cgtacctttt gtataatggg tcagcgactt acattcagtg gcagcttaac 600
    cgaataggga aggcgtcaga anagcagtcc gaatagggcg ttccagtcgc tgggtgtaga 660
     cccgaaacca gatgatctac ccatggccag gttgaaggca cggtaacacg tgctggagga 720
    ccgaacccac tagtgttgaa aaactagggg atgagctgtg gataggggtg aaaggctaaa 780
30
    caaatctqqa aataqctqqt tctctccqaa aactatttag gtagtgcctc aagtattact 840
    gcaggggta gagcactgtt atggctaggg ggtcatggcg acttaccaaa ccatggcaaa 900
    ctccgaatac ctgcaagtac agcttgggag acagacgacc gggtgctaac gtccggactc 960
    aagagggaaa caacccagac cgccagctaa ggtcccgaat tatcgctaag tgggaaacga 1020
    agtgggaagg catagacagt caggaggttg gcttagaagc agccaccctt taaagaaagc 1080
35
     qtaataqctc actgatcgag tcgtcctgcg cggaagatgt aacggctaag cgataaaccg 1140
     aaqctqcqqq tqtgcacttt tagtgcagcg gtaggagagc gttctgtaag cctgcgaagg 1200
     tgqcttqtaa aggctgctgg aggtatcaga agtgcgaatg ctgacatgag tagccataaa 1260
     gggggtgaaa agccccctcg ccgtaagtcc aaggtttcct gcgcaacgtt catcggcgca 1320
     gggtgagteg gecectaagg egaggeagag atgegtaget gatgggaage tggttaatat 1380
    tccagcaccg tcgtacagtg cgatgggggg acggatcgcg gaaggtcatc agggtgttgg 1440
     acgtccctgt tgctgcattg aagatggcgc ttaggcaaat ccgggcgcga gaatcaaggg 1500
     tgtggcacga gcgagcaagt ctcgcgaagt gattggaagt ggttccaaga aaagcctcta 1560
     agetteaget gtacqagace gtacegeaaa eegacacagg tgggaeggga tgaatattee 1620
     aaqqqqttg agagaactca ggagaaggaa ctcggcaaat tgataccgta acttcgggag 1680
45
     aaqqtatacc ctggtagtgt gaagcctgcg cgctgagcat gaaggggtcg cagagaatcg 1740
    gtggctgcga ctgtttatta aaaacacagc actctgcaaa gacgaaagtc gacgtatagg 1800
    gtgtgacgcc tgcccggtgc cggaaggtta agtgatgggg tgcaagctct tgatcgaagc 1860
     cccggtaaac ggcggccgta actataacgg tcctaaggta gcgaaattcc ttgtcgggta 1920
     agttccgacc tgcacgaatg gcgtaacgat ggccacactg tctcctcctg agactcagcg 1980
    aagttgaagt gtttqtqatq atgcaatcta cccqcggcta gacggaaaga ccccatgaac 2040
     ctttactgta gctttgcatt ggactgtgaa ccggcctgtg taggataggt gggaggcgca 2100
    gaactcgagt cgccagattc gagggagcca tccttgaaat accaccctgg tttgtttgcg 2160
    gttctaacct tggtccgtta tccggatcgg ggacagtgca tggtaggcag tttgactggg 2220
    geggteteet eccaaagegt aacggaggag ttegaaggta egetaggtae ggteggaaat 2280
55
    cgtgctgata gtgcaatggc ataagcgtgc ttgactgtga gactgacagt gaacaggtgc 2340
    gaacgggaca tagtgatccg gtggttctga tggaagggcc atcgctcaac ggataaaggt 2400
```

```
actotgggat aacaggotga tacogoccaa gagttcatat cgacggoggt gtttggcacc 2460
     tegatgtegg ctcatctcat cetggggetg tageeggtee aagggtatge tgttegeeat 2520
     ttaaagaggt acgtgagctg ggtttagaaa cgtcgtgaga cagtttggtc cctatctgcc 2580
     gtqqqcqttq qatacttqaa caqqaqcctq ctcctagtac gagaggaccg gagtggacgt 2640
     acctctqqtq taccqqttqt catqccaatq qcattqccqq qtaqctaagt acqqaaqaqa 2700
     taaccgctga aggcatctaa gcgggaaact cgtctgaaga ttaggtatcc cggggactag 2760
     ateccectqa agggtegtte gagaccagga egttgatagg tegggtgtgg aagegeagta 2820
     atgcgttaag ctaaccgata ctaattgccc gtgaggctta atcct
10
     <210> 64
     <211> 2865
     <212> DNA
     <213> Bordetella parapertussis
15
     -2205
     <221> modified base
     <222> (624)
     <223> N = A, C, G or T/U
20
     <400> 64
     qatcaagcga ctaagtgcat atggtggatg ccttggcgat cacaggcgat gaaggacgta 60
     gtagcctgcg aaaagctgcg gggagctggc aaacaagcat tgatccgcag atatccgaat 120
     ggggaaaccc acggcaagcg gtatccctgg ctgaatacat aggccagtgg aggcgaaccg 180
25
    ggtgaactga aacatctcag tagctcgagg aaaagaaatc aaccgagatt ccgaaagtag 240
    tggcgagcga aatcggaaga gcctttacga tttagcattt tgcatagtcg aacggaatgg 300
     aaagtccggc cgtagcaggt gatagccctg tagacgaaat gcagagtgtg gaactaggcg 360
     taagagaagt agggcgggac acgtgaaatc ctgtctgaag atggggggac catcctccaa 420
     ggctaaatac tcgtgatcga ccgatagtga accagtaccg tgaggaaagg cgaaaagaac 480
    cccqqaaqqa gtgaaataga tcctgaaacc gtatgcatac aaacagtcgg agcctcttta 540
30
     tggggtgacg gcgtaccttt tgtataatgg gtcagcgact tacattcagt ggcgagctta 600
     accgaatagg gaaggcgtca gaanagcagt ccgaataggg cgtccagtcg ctgggtgtag 660
     accegaaacc agatgateta eccatggeca ggttgaagge acggtaacac gtegtggagg 720
     accgaaccca ctagtgttga aaaactaggg gatgagctgt ggataggggt gaaaggctaa 780
35
    acaaatctqq aaataqctqq ttctctccqa aaactattta ggtagtgcct caagtattac 840
     tqcaqqqqqt agaqcactgt tatggctagg gggtcatggc gacttaccaa accatggcaa 900
     actocqaata cotgoaagta cagottggga gacagacgac cgggtgctaa cgtccggact 960
     caaqaggaa acaacccaga ccgccagcta aggtcccgaa ttatcgctaa gtgggaaacg 1020
     aagtgggaag gcatagacag tcaggaggtt ggcttagaag cagccaccct ttaaagaaag 1080
40
     cgtaataget cactgatega gtegteetge geggaagatg taaeggetaa gegataaace 1140
     gaagetgegg gtgtgcactt ttagtgcage ggtaggagag egttetgtaa geetgegaag 1200
     qtqqcttqta aaqqctqctq qaqqtatcaq aagtgcgaat gctgacatga gtagcgataa 1260
     agggggtgaa aagccccctc gccgtaagtc caaggtttcc tgcgcaacgt tcatcggcgc 1320
     agggtgagtc ggcccctaag gcgaggcaga gatgcgtagc tgatgggaag ctggttaata 1380
     ttccagcacc gtcgtacagt gcgatggggg gacggatcgc ggaaggtcat cagggtgttg 1440
     gacqtccctg ttgctgcatt gaagatggcg cttaggcaaa tccgggcgcg agaatcaagg 1500
     qtqtqqcacg agcgagcaag tctcgcgaag tgattggaag tggttccaag aaaagcctct 1560
     aagetteage tgtacgagae egtacegeaa acegacacag gtgggacggg atgaatatte 1620
     caaggogett gagagaacte aggagaagga acteggeaaa ttgatacegt aactteggga 1680
50
    gaaggtatac cctggtagtg tgaagcctgc gcgctgagca tgaaggggtc gcagagaatc 1740
     ggtggctgcg actgtttatt aaaaacacag cactctgcaa agacgaaagt cgacgtatag 1800
     ggtgtgacgc ctgcccggtg ccggaaggtt aagtgatggg gtgcaagctc ttgatcgaag 1860
     ccccggtaaa cggcggccgt aactataacg gtcctaaggt agcgaaattc cttgtcgggt 1920
     aagttccgac ctgcacgaat ggcgtaacga tggccacact gtctcctcct gagactcagc 1980
55
    gaagttgaag tgtttgtgat gatgcaatct accegeggct agacggaaag accecatgaa 2040
     cctttactgt agctttgcat tggactgtga accggcctgt gtaggatagg tgggaggcgc 2100
```

```
agaactcgag tegccagatt cgagggagec atecttgaaa taccaccetg gtttgtttgc 2160
     ggttctaacc ttggtccgtt atccggatcg gggacagtgc atggtaggca gtttgactgg 2220
     ggcggtctcc tcccaaagcg taacggagga gttcgaaggt acgctaggta cggtcggaaa 2280
     teqtqctqat aqtgcaatgg cataageqtq cttqactqtq agactgacag teqaacagqt 2340
     qcqaacqqqa cataqtqatc cqqtqqttct qatqqaaqqq ccatcqctca acqqataaaq 2400
     gtactctggg ataacaggct gataccgccc aagagttcat atcgacggcg gtgtttggca 2460
     cctcgatgtc ggctcatctc atcctggggc tgtagccggt ccaagggtat gctgttcgcc 2520
     atttaaagag gtacgtgagc tgggtttaga aacgtcgtga gacagtttgg tccctatctg 2580
     cogtogget togatactty aacaggage toctectagt acgagaggac cogagtogac 2640
10
    gtacctctqq tqtaccqqtt qtcatqccaa tqqcattqcc qqqtaqctaa qtacqqaaqa 2700
     gataaccgct gaaggcatct aagcggaaac tcgtctgaag attaggtatc ccgggactag 2760
     atcccctga agggtcgttc gagaccagga cgttgatagg tcgggtgtgg aagcgcagta 2820
     atgcqttaag ctaaccgata ctaattgccc gtgaggcttg atcct
15
     <210> 65
     <211> 2864
     <212> DNA
     <213> Bordetella pertussis
20
     <220>
     <221> modified base
     <222> (624)
     <223> N = A, C, G or T/U
25
     <400> 65
     gatcaagcga ctaagtgcat atggtggatg ccttggcgat cacaggcgat,gaaggacgta 60
     gtagcctgcg aaaagctgcg gggagctggc aaacaagcat tgatccgcag atatccgaat 120
     qqqqaaaccc acqqcaaqcq gtatccctqq ctqaatacat aqqccaqtqq aqqcqaaccq 180
30
     qqtqaactqa aacatctcag tagctcgagg aaaagaaatc aaccgagatt ccgaaagtag 240
     tgqcqagcqa aatcggaaga gcctttacga tttagcattt tgcatagtcg aacggaatgg 300
     aaagtccggc cgtagcaggt gatagccctg tagacgaaat gcagagtgtg gaactaggcg 360
     taagagaagt agggcgggac acgtgaaatc ctgtctgaag atggggggac catcctccaa 420
    ggctaaatac tcgtgatcga ccgatagtga accagtaccg tgaggaaagg cgaaaagaac 480
35
    cccggaagga gtgaaataga tcctgaaacc gtatgcatac aaacagtcgg agcctcttta 540
     tggggtgacg gcgtaccttt tgtataatgg gtcagcgact tacattcagt ggcgagctta 600
     accgaatagg gaaggcgtca gaanagcagt ccgaataggg cgtccagtcg ctgggtgtag 660
     accequance agatgateta cecatggeen ggttgangge aeggtanene gtegtggagg 720
    accqaaccca ctagtgttga aaaactaggg gatgagctgt ggatagggt gaaaggctaa 780
    acaaatctgg aaatagctgg ttctctccga aaactattta ggtagtgcct caagtattac 840
     tgcaggggt agagcactgt tatggctagg gggtcatggc gacttaccaa accatggcaa 900
     actecgaata cetgeaagta cagettggga gacagaegae egggtgetaa egteeggaet 960
     caaqaqqaa acaacccaqa ccqccaqcta aqqtcccqaa ttatcqctaa qtqqqaaacq 1020
     aagtgggaag gcatagacag tcaggaggtt ggcttagaag cagccaccct ttaaagaaag 1080
     cgtaatagct cactgatega gtegteetge geggaagatg taaeggetaa gegataaace 1140
     qaaqctqcqq qtqtqcactt ttaqtqcaqc qqtaqqaqaq cqttctqtaa qcctqcqaaq 1200
    qtqqcttqta aaqqctqctq qaqqtatcaq aaqtqcqaat qctqacatga qtaqcqataa 1260
     agggggtgaa aagccccctc gccgtaagtc caaggtttcc tgcgcaacgt tcatcggcgc 1320
    agggtgagtc ggcccctaag gcgaggcaga gatgcgtagc tgatgggaag ctggttaata 1380
50
    ttccagcacc gtcgtacagt gcgatggggg gacggatcgc ggaaggtcat cagggtgttg 1440
    gacgtccctg ttgctgcatt gaagatggcg cttaggcaaa tccgggcgcg agaatcaagg 1500
    gtgtggcacg agegagcaag tetegegaag tgattggaag tggttecaag aaaageetet 1560
    aagetteage tgtacgagac egtacegeaa acegacacag gtgggaeggg atgaatatte 1620
    caaggggett gagagaactc aggagaagga actcggcaaa ttgataccgt aacttcggga 1680
55
    qaaqqtatac cctqqtaqtq tqaaqcctqc qcqctqaqca tqaaqqqtc qcaqaqaatc 1740
    qqtqqctqcq actqtttatt aaaaacacaq cactctqcaa aqacqaaaqt cqacqtataq 1800
```

WO 03/054162 PCT/US02/41014

	gatataacac	ctacccaata	ccqqaaqqtt	aagtgatggg	gtgcaagete	ttgatcgaag	1860
						cttgtcgggt	
						gagactcagc	
						accccatgaa	
5						tgggaggcgc	
-						gtttgtttgc	
						gtttgactgg	
						cggtcggaaa	
						tcgaacaggt	
10						acggataaag	
						gtgtttggca	
						gctgttcgcc	
						ccctatctgc	
						ggagtggacg	
15						tacggaagag	
						cgggactaga	
						agcgcagtaa	
		taaccgatac				agogoagoaa	2864
	egogeeaage	caacegacae	Luadegeoog	0343300034			2002
20							
	<210> 66						
	<211> 2878						
	<212> DNA						
	<213> Burkh	nolderia cep	oacia				
25		•					
	<400> 66						
	ggtcaagcga	acaagtgcat	gtggtggatg	ccttggcgat	cacaggcgat	gaaggacgcg	60
						atgtccgaat	
	ggggaaaccc	actccttttg	gagtatccat	ggctgaatac	ataggccatg	cgaaggaacg	180
30	cggtgaactg	aaacatctaa	gtaaccgcag	gaaaagaaat	caaccgagat	tcccaaagta	240
	gtggcgagcg	aaatgggatg	agccttgcac	tctttatttg	tattgttagc	cgaacgctct	300
	ggaaagtgcg	gccatagcag	gtgatagccc	tgtaggcgaa	aacagtatga	aagaactagg	360
	tgtgcgacaa	gtagggcggg	acacgtgaaa	tcctgtctga	agatgggggg	accatcctcc	420
	aaggctaaat	actcgtgatc	gaccgatagt	gaaccagtac	cgtgagggaa	aggcgaaaag	480
35	aaccccggga	ggggagtgaa	atagatcctg	aaaccgcatg	catacaaaca	gtcggagcct	540
	cgtaaggggt	gacggcgtac	cttttgtata	atgggtcagc	gacttacgtt	cagtagcaag	600
	cttaaccgta	tagggcaggc	gtaggaaagg	agtccgaata	gggcgttcag	ttgctgggcg	660
	tagacccgaa	accaggtgat	ctatccatgg	ccaggatgaa	ggtgcggtaa	cacgtactgg	720
	aggtccgaac	ccactaacgt	tgaaaagtta	ggggatgagc	tgtggatagg	ggtgaaaggc	780
40	taaacaaacc	tggaaatagc	tggttctctc	cgaaaactat	ttaggtagtg	cctcgtgtct	840
	caccttcggg	ggtagagcac	tgtcatggtt	ggggggtcta	ttgcagatta	ccccgccata	900
	gcaaactccg	aataccgaag	agtgcaatca	cgggagacag	acatcgggtg	ctaacgtccg	960
	gtgtcaagag	ggaaacaacc	cagaccgcca	gctaaggtcc	ccaaatatag	ctaagtggga	1020
						ccctttaaag	
45	aaagcgtaat	agctcactga	tcgagtcgtc	ctgcgcggaa	gatgtaacgg	ggctaagcta	1140
	tataccgaag	ctgcggatgc	gtgctttgca	cgatggtagg	agagcgttcc	gtaagcctgc	1200
						atgagtagcg	
	ataaaggggg	tgaaaggccc	cctcgccgta	agcccaaggt	ttcctacgca	acgttcatcg	1320
						gaagcaggtc	
50						ttgtccgggt	
	gttggaagtc	ccggtcgctg	cattggagaa	ggcgcttagg	caaatccggg	cgcagaattc	1500
						aagaaaagcc	
						gagatgagta	
						cgtaacttcg	
55						gggttgcaat	
						aaagtggacg	
	33 33			-	-		

WO 03/054162 PCT/US02/41014

	t at agget at	ascactace	cggtgccgga	agattaaatg	atggggtgca	acctettest	1860
			gccgtaacta				
			cgaatggcgt				
			gtgatgatgc				
5			tgcattggac				
			agtttcggtg				
			ccgtgatccg				
			aagcgtaacg				
			aatggcataa				
10			tagtgatccg				
			gataacaggc				
			cggctcatct				
			gaggtacgtg				
			gttggatatt				
15			tgtaccggtt				
			gaaagcatct				
			tgaagggtcg				
			cagctaactg				2878
	-55-5	3			3		
20							
	<210> 67						
	<211> 2882						
	<212> DNA						
	<213> Burkl	holderia mal	llei				
25							
	<400> 67						
	ggtcaagcga	acaagtgcat	gtggtggatg	ccttggcgat	cacaggcgat	gaaggacgcg	60
	gtagcctgcg	aaaagctacg	gggagctggc	aaacgagctt	tgatccgtag	atgtccgaat	120
	ggggaaaccc	ggcccttttg	ggtcatccta	gactgaatac	ataggtctag	tgaggcgaac	180
30	gcggtgaact	gaaacatcta	agtaaccgca	ggaaaagaaa	tcaaccgaga	ttcccaaagt	240
	agtggcgagc	gaaatgggaa	gagcctgtac	tctttatttg	tattgttagc	cgaacgctct	300
	ggaaagtgcg	gccatagcag	gtgatagccc	tgtaggcgaa	aacagtatga	aagaactagg	360
			acacgtgaaa				
			gaccgatagt				
35			atagatcctg				
			cttttgtata				
			gtagcgaaag				
			atctatccat				
40			gttgaaaagt				
40			gctggttctc				
			actgtcatgg				
			agagtgcaat				
			cccagaccgc				
			aaacagtcag				
45			gatcgagtcg				
			gcgagctagt				
			cgtgctggag				
			cccctcgcc				
			ccctaaggcg				
50			gttagatgcg				
			ctgcattgga				
			tccttcggga				
			cgatgaccgt				
			gaactcggga				
55			gtagcttgac				
	aataaactgg	tggctgcgac	tgtttaataa	aaacacagca	ctctgcaaac	acgaaagtgg	1800

```
acgtataggg tgtgacgcct gcccggtgcc ggaagattaa atgatggggt gcaagctctt 1860
     gattgaagtc ccggtaaacg gcggccgtaa ctataacggt cctaaggtag cgaaattcct 1920
     tgtcgggtaa gttccgacct gcacgaatgg cgtaacgatg gccacactgt ctcctcccga 1980
     gactcagcga agttgaagtg tttgtgatga tgcaatctac ccgcggctag acggaaagac 2040
    cccatgaacc tttactgtag ctttgcattg gactttgaac cgatctgtgt aggataggtg 2100
     qqaqqctatq aaaccqqaat qctaqtttcg qtggaqccqt ccttqaaata ccaccctggt 2160
     ttgtttqaqq ttctaacctt qqccqtqat ccgggtcgqq gacagtqcat ggtagqcagt 2220
     ttgactgggg cggtctcctc ccaaagcgta acggaggagt acgaaggtac gctaggtacg 2280
     gteggaaate gtgetgatag tgeaatggea taagegtget taactgegag accgacaagt 2340
10
    cgagcaggtg cgaaagcagg tcatagtgat ccggtggttc tgtatggaag ggccatcgct 2400
     caacggataa aaggtactct ggggataaca ggctgatacc gcccaagagt tcatatcgac 2460
     qqqqtqttt qqcacctcqa tqtcqqctca tctcatcctq qqqctqtaqc cqgtcccaaq 2520
     qqtatqqctq ttcqccattt aaaqaqgtac gtqaqctqqq tttaaaacqt cgtgagacag 2580
     tttqqtccct atctqccqtq qqcgttggaa gtttgaaggg ggctgctcct agtacgagag 2640
15
    qaceggagtg gacgaacete tggtgtaceg gttgtgacge cagtegcate geegggtage 2700
     tatgttcgga agagataacc gctgaaagca tctaagcggg aaactcgcct taagatgaga 2760
     cttccccggg gacttgatcc ccttgaaggg tcgttcgaga ccaggacgtt gataggtcgg 2820
     gtgtgtaage geagtaatge gtteagetaa eegataetaa ttgeeegtae ggettgatee 2880
20
     <210> 68
     <211> 2882
     <212> DNA
25
    <213> Burkholderia pseudomallei
     <400> 68
     ggtcaagcga acaagtgcat gtggtggatg ccttggcgat cacaggcgat gaaggacgcg 60
     gtagcctgcg aaaagctacg gggagctggc aaacgagctt tgatccgtag atgtccgaat 120
30
     ggggaaaccc ggcccttttg ggtcatccta gactgaatac ataggtctag tgaggcgaac 180
     qcggtgaact gaaacatcta agtaaccgca ggaaaagaaa tcaaccgaga ttcccaaagt 240
     agtqqcqaqc qaaatqqqaa qaqcctqtac tctttatttq tattqttaqc cqaacqctct 300
     ggaaagtgcg gccatagcag gtgatagccc tgtaggcgaa aacagtatga aagaactagg 360
    tgtacgacaa gtagggcggg acacgtgaaa tcctgtctga agatgggggg accatcctcc 420
35
     aaggctaaat actcgtgatc gaccgatagt gaaccagtac cgtgagggaa aggcgaaaag 480
     aaccccqqqa qqqqaqtqaa atagatcctq aaaccgcatq catacaaaca gtcqgaqcct 540
     cttcgqqqqt qacqqcqtac cttttgtata atqqqtcagc gacttacqtt cagtaqcaaq 600
     cttaaccgaa tagggcaggc gtagcgaaag cgagtccgaa tagggcgttc agttgctggg 660
    cgtagacccg aaaccaggtg atctatccat ggccaggatg aaggtgcggt aacacgtact 720
40
    ggaggtccga acccactaac gttgaaaagt taggggatga gctgtggata ggggtgaaag 780
     gctaaacaaa cctggaaata gctggttctc tccgaaaact atttaggtag tgcctcgtgt 840
     ctcaccttcg ggggtagage actgtcatgg ttggggggtc tattgcagat taccccgcca 900
     taqcaaactc cqaataccqa aqaqtqcaat cacqqqaqac aqacatcqqq tqctaacqtc 960
     cqqtqtcaaq aqqqaaacaa cccaqaccqc caqctaaqqt ccccaaatat qqctaaqtqq 1020
    qaaacqaaqt qqqaaqqcta aaacaqtcaq qaqqttqqct taqaaqcaqc caccctttaa 1080
     agaaagcqta atagctcact gatcqaqtcg tcctgcqcgg aagatqtaac qgggctaagc 1140
     catataccya agctgcqqat qcgagctagt ctcgcatggt aggagagcgt tccgtaagcc 1200
     tgcgaaggtg cgttgaaaag cgtgctggag gtatcggaag tgcgaatgct gacatgagta 1260
     gcgataaagg gggtgaaagg ccccctcgcc gtaagcccaa ggtttcctac gcaacgttca 1320
50
    tcgqcgtagg gtqagtcggc ccctaaggcg aggcagaaat gcgtagctga tgggaagcag 1380
    gtcaatattc ctgcaccgtc gttagatgcg atggggggac ggatcgcgga aggttgtccg 1440
    ggtgttggaa gtcccggtcg ctgcattgga gaaggcgctt aggcaaatcc gggcgcagga 1500
    ttcaagggtg tggcgcgagc gctctagggc gcgaagcaat tggaagtggt tccaagaaaa 1560
    qcctctaaqc ttcaqtctaa cqatqaccqt accqcaaacc qacacaqqtq qqcqaqatqa 1620
    qtattctaaq qcqcttqaqa gaactcggga qaaggaactc qgcaaattqq taccgtaact 1680
    tegggataaq qtaegeeetg gtagettgae tggeetqege cagaaqqqtg aaggggttge 1740
```

43/52

```
aataaactgg tggctgcgac tgtttaataa aaacacagca ctctgcaaac acgaaagtgg 1800
    acgtataggg tgtgacgcct gcccggtgcc ggaagattaa atgatggggt gcaagctctt 1860
    gattgaagtc ccggtaaacg gcggccgtaa ctataacggt cctaaggtag cgaaattcct 1920
    tgtcgggtaa gttccgacct gcacgaatgg cgtaacgatg gccacactgt ctcctcccga 1980
    gactcagcga agttgaagtg tttgtgatga tgcaatctac ccgcggctag acggaaagac 2040
    cccatgaacc tttactgtag ctttgcattg gactttgaac cgatctgtgt aggataggtg 2100
    qqaqqctatq aaaccqqaac gctagtttcg gtgqaqccgt ccttgaaata ccaccctggt 2160
    ttgtttgagg ttctaacctt ggcccgtgat ccgggtcggg gacagtgcat ggtaggcagt 2220
    ttgactgggg cggtctcctc ccaaagcgta acggaggagt acgaaggtac gctaggtacg 2280
    qtcggaaatc gtgctgatag tgcaatggca taagcgtgct taactgcgag accgacaagt 2340
    cgagcaggtg cgaaagcagg tcatagtgat ccggtggttc tgtatggaag ggccatcgct 2400
    caacggataa aaggtactct ggggataaca ggctgatacc gcccaagagt tcatatcgac 2460
    ggcggtgttt ggcacctcga tgtcggctca tctcatcctg gggctgtagc cggtcccaag 2520
    gqtatqqctq ttcqccattt aaaqaqqtac gtgagctggg tttaaaacgt cgtgagacag 2580
15
    tttggtccct atctgccgtg ggcgttggaa gtttgaaggg ggctgctcct agtacgagag 2640
    gaccggagtg gacgaacctc tggtgtaccg gttgtgacgc cagtcgcatc gccgggtagc 2700
    tatgttegga agagataace getgaaagea tetaageggg aaactegeet taagatgaga 2760
    cttccccggg gacttgatcc ccttgaaggg tcgttcgaga ccaggacgtt gataggtcgg 2820
    gtgtgtaagc gcagtaatgc gttcagctaa ccgatactaa ttgcccgtac ggcttgatcc 2880
20
                                                                       2882
    <210> 69
    <211> 2890
25
    <212> DNA
    <213> Neisseria gonorrhoeae
    <400> 69
    ggtcaagtga ataagtgcat caggcggatg ccttggcgat gataggcgac gaaggacgtg 60
    taagcctgcg aaaagcgcgg gggagctggc aataaagcta tgattccgcg atgtccgaat 120
    ggggaaaccc actgcattct gtgcagtatc ctaagttgaa tacataggct tagagaagcg 180
    aacccqqaqa actgaaccat ctaagtaccc ggaggaaaag aaatcaaccg agattccgca 240
    aqtaqtqqcg agcgaacgcg gaggagcctg tacgtaataa ctgtcgagat agaagaacaa 300
    qctqggaagc ttgaccatag cgggtgacag tcccgtattc gaaatctcaa cagcggtact 360
35 aagcgtacga aaagtagggc gggacacgtg aaatcetgte tgaatatggg gggaccatec 420
    tccaaggcta aatactcatc atcgaccgat agtgaaccag taccgtgagg gaaaggcgaa 480
    aagaaccccg ggagggaagt gaaacagaac ctgaaacctg atgcatacaa acagtgggag 540
    cgccctagtg gtgtgactgc gtaccttttg tataatgggt caacgactta cattcagtag 600
    cqaqcttaac cqqatagggg aggcgtaggg aaaccgagtc ttaatagggc gatgagttgc 660
    tgggtgtaga cccgaaaccg agtgatctat ccatggtcag gttgaaggtg ccgtaacagg 720
    tactgqagga ccgaacccac gcatgttgca aaatgcgggg atgagctgtg ggtaggggtg 780
    aaaggctaaa caaactcgga gatagctggt tctccccgaa aactatttag gtagtgcctc 840
    gagcaagaca ctgatggggg taaagcactg ttatggctag ggggttattg caacttacca 900
    acccatggca aactcagaat accatcaagt ggttcctcgg gagacagaca gcgggtgcta 960
45
    acgtccgttg tcaagaggga aacaacccag accgccggct aaggtcccaa atgatagatt 1020
    aagtggtaaa cgaagtggga aggcacagac agccaggatg ttggcttaga agcagccatc 1080
    atttaaagaa agcgtaatag ctcactggtc gagtcgtcct gcgcggaaga tgtaacgggg 1140
    ctcaaatcta taaccgaagc tgcggatgcc ggtttaccgg catggtaggg gagcgttctg 1200
     taggctgatg aaggtgcatt gtaaagtgtg ctggaggtat cagaagtgcg aatgttgaca 1260
    tgagtagcga taaagcgggt gaaaagcccg ctcgccgaaa gcccaaggtt tcctacgcaa 1320
    cqttcatcqq cqtaqggtaa gtcggccct aaggcgaggc agaaatgcgt agtcgatggg 1380
     aaacaqqtta atattcctgt acttgattca aatgcgatgt ggggacggag aaggttaggt 1440
    tggcaagctg ttggaatagc ttgtttaagc cggtaggtgg aagacttagg caaatccggg 1500
    ttttcttaac accgagaagt gatgacgagt gtctacggac acgaagcaac cgataccacg 1560
55
    cttccaggaa aagccactaa gcttcagttt gaatcgaacc gtaccccaaa ccgacacagg 1620
```

tgggtaggat gagaatteta aggegettga gagaactegg gagaaggaac teggeaaatt 1680

```
gataccgtaa cttcgggaga aggtatgccc tctaaggtta aggacttgct ccgtaagccc 1740
     cggagggtcg cagagaatag gtggctgcga ctgtttatta aaaacacagc actctgccaa 1800
     cacqaaaqtq qacqtataqq qtqtqacqcc tqcccqqtqc cggaaggtta attgaagatg 1860
    tqcaaqcatc qqatcqaaqc cccqqtaaac gqcqqccqta actataacgg tcctaaggta 1920
    gcgaaattcc ttgtcgggta agttccgacc cgcacgaatg gcgtaacgat ggccacactq 1980
     tetecteceg agacteageg aagttgaagt ggttgtgaag atgeaateta eeegetgeta 2040
    gacqqaaaqa ccccgtgaac ctttactgta gctttgcatt ggactttgaa gtcacttgtq 2100
    taggataggt gggaggettg gaagcagaga egecagtete tgtggagteg teettgaaat 2160
    accaccotgg tgtctttgag gttctaaccc agacccgtca tccgggtcgg ggaccgtgca 2220
10
    tggtaggcag tttgactggg geggteteet cccaaagegt aacggaggag ttcgaaggtt 2280
    acctaggtcc ggtcggaaat cggactgata gtgcaatggc aaaaggtagc ttaactgcga 2340
    gaccgacaag tcgggcaggt gcgaaagcag gacatagtga tccggtggtt ctgtatggaa 2400
    qqqccatcqc tcaacqqata aaaggtactc cggggataac aggctgattc cgcccaagag 2460
    ttcatatcga cggcggagtt tggcacctcg atgtcggctc atcacatcct ggggctgtag 2520
15 teggteccaa gggtatgget gttegecatt taaagtggta cgtgagetgg gtttaaaacg 2580
    tegtgagaca gtttggteec tatetgeagt ggegttggaa gtttgaeggg getgeteeta 2640
    gtacgagagg accggagtgg acgaacctct ggtgtaccgg ttgtaacgcc agttgcatag 2700
    cogggtaget aagttoggaa qaqataaqog otgaaagcat otaagogoga aactogootg 2760
    aaqatqaqac ttcccttqcq qtttaaccqc actaaaqqqt cqttcgagac caggacqttg 2820
20
    ataggtgggg tgtggaagcg cggtaacgcg tgaagctaac ccatactaat tgcccgtgag 2880
                                                                     2890
    gcttgactct
    <210> 70
25
    <211> 2891
     <212> DNA
    <213> Neisseria meningitidis
    <400> 70
30
    qtcaaqtqaa taagtqcatc aggtggatgc cttggcgatg ataggcgacg aaggacgtgt 60
    aagcctgcga aaagcgcggg ggagctggca ataaagcaat gatcccgcga tgtccgaatg 120
    gggaaaccca ctgcattctg tgcagtatcc taagttgaat acatagactt agagaagcga 180
    accoggagaa ctgaaccatc taagtacccg gaggaaaaga aatcaaccga gattccgcaa 240
    gtagtggcga gcgaacgcgg aggagcctgt acgtaataac tgtcgagata gaagaacaag 300
    ctgggaaget tgaccatagt gggtgacagt cccgtattcg aaatetcaac agcggtacta 360
    agcgtacgaa aagtagggcg gggcacgtga aatcctgtct gaatatgggg ggaccatcct 420
    ccaaqqctaa atactcatca tcqaccqata gtgaaccagt accgtgaggg aaaggcgaaa 480
    aqaacccqq qaqqqqaqtq aaacaqaacc tqaaacctga tgcatacaaa cagtgggagc 540
    gccctagtgg tgtgactgcg taccttttgt ataatgggtc aacgacttac attcagtagc 600
    gagettaace gaatagggga ggegtaggga aacegagtet taatagggeg atgagttget 660
     gggtgtagac ccgaaaccga gtgatctatc catggccagg ttgaaggtgc cgtaacaggt 720
     actggaggac cgaacccacg catgttgcaa aatgcgggga tgagctgtgg ataggggtga 780
    aaggctaaac aaacteggag atagetggtt eteecegaaa actatttagg tagtgeeteg 840
    agcaagacac tgatgggggt aaagcactgt tatggctagg gggttattgc aacttaccaa 900
45
    cccatggcaa actaagaata ccatcaagtg gttcctcggg agacagacag cgggtgctaa 960
     cqtccqttqt caaqaqqqaa acaacccaqa ccqccaqcta agqtcccaaa tgatagatta 1020
     agtggtaaac gaagtgggaa ggcccagaca gccaggatgt tggcttagaa gcagccatca 1080
     tttaaaqaaa qoqtaatage tcactggtog agtogtootg ogoggaagat gtaacggggc 1140
    tcaaatctat aaccqaaqct gcqgatgccg gtttaccggc atggtagggg agcgttctgt 1200
50
     aggetgatga aggtgeattg taaagtgtge tggaggtate agaagtgega atgttgacat 1260
    gagtagcgat aaagcgggtg aaaagcccgc tcgccgaaag cccaaggttt cctgcgcaac 1320
    qttcatcggc gtagggtgag tcggccccta aggcgaggca gaaatgcgta gtcgatggga 1380
    ggcaagctgt tggaatagct tgtttaagcc ggtaggtgga agacttaggc aaatccgggt 1500
55
    cttettaaca ceqaqaaqtq acqaeqaqtq tetacqqaca eqaaqcaace qataccaege 1560
     ttccaqqaaa aqccactaaq cttcaqtttq aatcqaaccq taccqcaaac cgacacaggt 1620
```

PCT/US02/41014 45/52

		agaattataa	aggasttaga	agaactcagg	agaaggaact	cggcaaattg	1680
						cgtaagcccc	
						ctctgctaac	
						ttgaagatgt	
5						cctaaggtag	
-						gccacactgt	
						ccgctgctag	
						tcacttgtgt	
						ccttgaaata	
10						gaccgtgcat	
						tcgaaggtta	
						taactgcgag	
						tgtatggaag	
						gcccaagagt	
15						gggctgtagt	
						tttaaaacgt	
	cgtgagacag	tttggtccct	atctgcagtg	ggcgttggaa	gtttgacggg	ggctgctcct	2640
						cagttgcata	
	gccgggtagc	taagttcgga	agagataagc	gctgaaagca	tctaagcgcg	aaactcgcct	2760
20	gaagatgaga	cttcccttgc	ggtttaaccg	cactaaagag	tcgttcgaga	ccaggacgtt	2820
	gataggtggg	gtgtggaagc	gcggtaacgc	gtgaagctaa	cccatactaa	ttgctcgtga	2880
	ggcttgactc	t					2891
20							
25	<210> 71						
	<211> 2891						
	<212> DNA						
		domonas aem	vainosa				
		domonas aer	ıginosa				
30		domonas aer	ıginosa				
30	<213> Pseud		_	ccttggcagt	cagaggcgat	gaaagacgtg	60
30	<213> Pseud <400> 71 ggtcaagtga	agaagcgcat	acggtggatg			gaaagacgtg atctctgaat	
30	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg	agaagcgcat aaaagcttcg	acggtggatg gggagtcggc	aaacagactt	tgatccggag	atctctgaat	120
30	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc	agaagcgcat aaaagcttcg acctaggata	acggtggatg gggagtcggc acctaggtat	aaacagactt cttgtactga	tgatccggag atccataggt		120 180
30 35	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct	aaacagactt cttgtactga tgaggaaaag taagcttcat	tgatccggag atccataggt aaatcaaccg tgattttagc	atctctgaat gcaagaggcg agattccctt ggaacgctct	120 180 240 300
	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaaagtgcg	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtgg	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgatagccc	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc	120 180 240 300 360
	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaaagtgcg ggaagtaggacg	agaagcgcat aaaagcttcg acctaggata actgaacg ggcatagtgg gccatagtgg gagcacgaga	acggtggatg gggagtcggc acctaggtat ctaagtacc gatagccct gtgatagccc aactttgtct	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa	120 180 240 300 360 420
	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaaagtgcg ggaaagtgcg gagaaggacg atactactga	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtgg gagcacgaga ctgaccgata	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgatagccc aactttgtct gtgaaccagt	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaacccgg	120 180 240 300 360 420 480
35	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaaagtgcg gagtaggacg atactactga agaggggagt	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtgg gagcacgaga cgaccgata gaaatagaac	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgatagccc aactttgtc gtgaaccagt ctgaaaccgt	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atgcgtacaa	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt	120 180 240 300 360 420 480 540
	<213> Pseudo <400> 71 ggtcaagtga gtagcetgeg gggggaacce aaccagggga agtagtggeg ggaaagtgeg gagtagtgeg gagtagtgeg agaggggagt agaggggagt aggtgactge	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtgg gagcacgaga ctgaccgata gaaatagaac gtaccttttg	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccc gtgatagccc actttgtct gtgaaccagt ctgaaaccgt tataatgggt	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atgcgtacaa cagcgactta	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac	120 180 240 300 360 420 480 540 600
35	<213> Pseudo <400> 71 ggtcaagtga gtagcctgog gggggaaccc aaccagggga agtagtggog gagtagtggog gagtaggacg atactactga agagggagt aggtgactgo cgtataggc gtataggc	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gcatagtgg gagcacgaga ctgaccgata gaaatagaac gtaccttttg	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgatagccc aactttgtct gtgaaccagt ctgaaccagt ctgaaaccgt taaatgggt	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atgcgtacaa cagcgactta ttaatagggc	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtggag tattcagtgg gtttagtcgc	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataga	120 180 240 300 360 420 480 540 600 660
35	<113> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtgcg ggaaagtgcg ggaagtgcg atactactga agaggggagt aggtgactgc ccgtataggac ccgtataggac	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtgg gagcacgaga ctgaccgata gaaatagaac gtaccttttg aggcgtagcg	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgatagccc accttgtct tgaaaccagt ctgaaaccgt tataatgggt aaagcgagtc ccatgagcag	aaacagactt cttgtactga tgaggaaaaa taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atgcgtacaa cagcgactta ttaatagggc gttgaaggtt	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg gtttagtcgc aggtaacact	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataga gactggagga	120 180 240 300 360 420 480 540 600 660 720
35	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaaagtggcg ggaaagtggcg atactactga agaggggagt aggtgaggcc ccgaaccg ccgaaacca	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtgg gagcacgaga ctgaccgata gaaatagaac gtaccttttg agcgtagcg ggcgatctat tcccgttgaa	acggtggatg gggagtcggc acctaggtat tctaagtaccc gattagccct gtgatagccc stgaaaccagt ctgaaaccagt ctgaaaccgg tataaatggg aaaggagtc ccatgagcag aaggtaggaga	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg atgcgtacaa cagcgactta ttaatagggc gttgaaggt atgaatgtta	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg gtttagtcgg aggtaacact gatcggagtg	atctctgaat gcaagaggc agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataac tgggtataga aaaggctaat	120 180 240 300 360 420 480 540 600 660 720 780
35 40	<113> Pseudo <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtgcg ggaaagtgcg ggaagtgcg gatactactga agaggggagt aggtgactgc cgtatagggc ccgaaaccca ccaagctcgga	agaagcgcat aaaagcttcg acctaggata actgaaacat agcgaacggg gccatagtg gagcacgata ctgaccgata gaaatagaac gtaccttttg agcgtagcg ggcgatctat tcccgttgaa	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgaaagccc accttgtct gtgaaccagt ctgaaaccgt tataatgggt tataatgggt ccatgagcag aaggtagggg aaggtagggg	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gacattgaggg accgtgaggg atgcgtacaa cagegactta ttaatagggc gttgaaggtt atgacttgtgaggttagactta	tgatccggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtcggag tattcggag gtttagtcgc aggtaacact gatcggagt gtacgcact gatcggagt	atctctgaat gcaagaggc agattccctt ggaacgctct aagtgaaat ccaaggctaa agaacccgg cctacttgtt caagcttaac tgggtataga gactggagga aaaggctaat atgtatcact	120 180 240 300 360 420 480 540 660 720 780 840
35	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagsgga agtagtggcg ggataggacg agtaggacg agtaggacg actactactga agagggagat ccgaatagggc ccgaacccac ccgaacccac caggctcgga	agaagcgcat aaaagcttcg acctaggata actgaaacag gccatagtgg gagcacggg ctgaccggat ctgaccgata gaaatagaac gtaccttttg aggcgtagcg ggcgatctat tcccgttgaa gatagctggt	acggtggatg gggagtcggc acctaggtat ctaagtacc gattagccct gtgatagccc actttgtct gtgaaccgt ttgaaccgt tataatgggt aaagcgagtc ccatgagcag acgtaggag acgtagggg tcctctgaa	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atgcgtacaa cagcgactta ttaataggg gttgaaggtt atgacttgtg agctatttag ggtcattcag	tgatcoggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg gtttagtcgc aggtaacact gatcggagtg gtagcgctc acttaccaaa	atctctgaat gcaagaggcg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataga gactggagga aaaggctaat atgtatcact ccgatgcaaa	120 180 240 300 360 420 480 540 660 720 780 840 900
35 40	<pre><213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccaggga agtagtgcg ggaaagtagtgcg ggaaagtgcg gagtaggacg atactactga aggtgactgc cgtatagggt ccgaaacca cqaaccac caagctcgga ctggggggta</pre>	agaagcgcat aaaagcttcg acctaggata actggaacagg gcgaacggg ggccacagaga ctgaccgata gaaatagaac gtaccttttg ggcgatctat tcccgttgaa gatagctggt gagcactgt gacaactgt	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct gtgatagccc acctttgtct gtgaaccagt ctgaaaccgt tataatggg tataatggg ccatgagcag ccatgagcag tcgatgagcag tcgctaggg tctcctcgaa tcggataggg	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atgcgtacaa cagcgactta ttaatagggc gttgaaggtt atgacttetg agctatttag ggtcatcccg agacaccgg	tgatcoggag aatccataggt aaatcaaccg tgattttagc aggatctttag ggaccatcct aaaggcgaaa tattcagtgg gtttagtcgc aggtaacact gatggaacct gatggaacact gatagcgctca acttaccaaa	atctctgaat gcaagagggg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataag gactggagga aaaggctaat atgtatcact ccgatgcaaa	120 180 240 300 360 420 480 540 660 720 780 840 900 960
35 40	<213> Pseud <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaagtggcg ggataggacg agtagtgacg agagggagt aggtgactgc cgtatagggt cccgaaaccca ccaagctcgga ctggggggta ctccgaatca ctcggatag cgaaagggag agaaggggggg	agaagcgcat aaaagcttcg acctaggata actgaacat agcgaacgg gcatagtg gagcacgata gagcacgata gagcatttttg agcgctagcg ggcgatctat tcccgttgaa gatagctggt cagaactgt ccagaagtgc acaacccaga	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct ggatagccc tggatagccc tcgaaccagt ctgaaccagt ctgaaccagt ccatgagca aaggtaggg tccctcgaa aggtaggg ccatgagca	aaacagactt cttgtactga tgaggaaaag taagctcat cgtacgcgaa gaccatgggg accgtsaggg atgcgtacaa cagcgactta ttaataggc gttgaaggt atgaatttga ggtcatcccg agacaccgg aggcccaaa	tgatcoggag aatcaataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggggaa tattcagtgg gtttagtcgc aggtaacact gatcggagt taggcctc acttaccaaa cgggtyctaa gttgtggtc	atctctgaat gcaagaggg agattccctt ggaacgctct aagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataga gactgagga aaaggctaa atgtatcact ccgatgcaaa cgtcgaega agtggaaa	120 180 240 300 360 420 480 540 660 720 780 840 900 960 1020
35 40	<pre><213> Pseuco <400> 71 ggtcaagtga gtagcctgcg gggggaaccc aaccagggga agtagtggcg ggaagtgggg ggaagtggcg cgtataggact cgtatagggc ccgaacca ccaagctcgga ctggaagtcgaact ccgaaacca ccaagctcgga ctggggggta ctgggagta ggtggggggga ctgggagta ggagggggggggg</pre>	agaagcgcat aaaagcttcg acctaggata actggaacggg gccatagtgg gccatagtgg gagcacgaga gtaacttttg aggcgtagcg gtaccttttg aggcgtagcg gccgttgaa gatagctggt cagcactgt cagaagtgc acaacccaga ggcttagac	acggtggatg gggagtcggc cactaggtat ctaagtacc gattagccct gtgatagccc aactttgtct gtgaaccagt ctgaaaccggt tataatgggt aaaggtagtgc ccatgagcag ccttgagcag cggcatggg cggcagcta	aaacagactt cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg accgtgaggg atggtacaa cagcgactta ttaatagggc gttgaaggt atgacttgtg agctatttag ggtcattattag ggcactacca agacacacgg aggtcccaaa tggcttaagaa	tgatcoggag atccataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggggaaa cattcagtgg gtttagtcgc aggtaacact gatcggagt gtagggggt aatcaggggtg tatccaaa cgggtgctaa gttgtgggt	atctctgaat gcaagagggg agattccctt ggaacgctct caaggctaa ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataga gactgagga aaaggctaat atgtatcact ccgatgcaaa cgtccgtcgt agtggtaaa ctttaaa	120 180 240 300 360 420 480 540 660 720 780 840 960 1020 1080
35 40 45	<213> Pseuc <400> 71 gytcaagtga gytgaagtga gyggggaaccc gagtagtggga gyaaagtgcgga gatactactga agtaggaagt agtaggaagt agtagtggg atactactga agagggagt cgtataggacg ccgaacccac ccgaacccac ccgaacccac cagottgga ctcggaatcc ctcgaatca ctcgaataggaga agtggaggaga attgggggta ctccgaatag cyaaagggaa agtgtagtggga cytaataggaa ggtaataggaa ggtaataggaa ggtaataggaa ggtaataggaa ggtaataggaa ggtaataggaa ggtaataggaa ggtaatatagc	agaagcgcat aaaagcttcg acctaggata actgagacat aggaacat aggaacagg gacatagtg gagcacgaga ctgaccgata gagactgatgg ggcgatctat tcccgttgaa gatagctggt gagaactgt ccagaagtgg ggcgatcagt gagcactgt ccagaagtgg ccagaactgg ggcttagaca	acggtggatg gggagteggc acctaggtat ctaagtacc gattagccct gtgatagccc ttgaacagt ctgaacagt ctgaacagt aaagcgagtc aaagtgaggag ctccctcgaa tcgctagga cgagcatggg cgagcatggg cgcagcaggg cgcagcagga	aaacagacttcat chtgtactga tgaggaaaag taagctcat cgtacggaa gaacatgggg atgcgtacaa cagcgactta ttaataggg ttgaaggt ttgaaggtt atgactttgt agctatttag ggcattcaaa cggcgacta atgactttga ggtcatcca aggcactacaa ggtcatcaca ggcactacacg aggacacacg	tgatcoggag atcataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg gtttagtcgc aggtaacact gatcggagtg gttagccctc acttaccaaa cgggtgctaa gttgtgcta ggttggtta gcagcaccc ggagtgctaa gttgtggtta gcagcaccc	atctctgaat gcaagaggg agattcctt ggaacgctct caagtgaaatc ccaaggttaa agaaccccg cctacttgtt caagcttaac tgggtataga gactgagga aatggagtaat atgtatcact ccgatgcaaa agtccgtcgt agtggtaaac tttaaagaaa	120 180 240 360 420 480 540 660 720 780 840 900 960 1020 1140
35 40	<pre><213> Pseuc <400> 71 ggtcaagtga gtagcctgeg gggggaaccc aaccagggga agtagtgcg ggaaatgcc ggatactactga agaggggag tactacttga agaggggag cccgaaaccc cccgaaaccc ccaagctggg ctggggggta ctccggaatac gaaaaggggag ctggggggta ctggggggta ctgggaggta ctgggaggggag</pre>	agaagcgcat aaaagcttcg acctaggata actgaacagt gccatagtgg gccatagtgg gagcacgaga ctgaccgaga ctgaccttttg aggcgtagcg ggcgatctat aggcgtagcg gatcactgtt gagcactgtt gagcactgtc cagaagtgc acaaccaga ggcttagac gcctagac gcctagac gcctagac	acggtggatg gggagtcggc acctaggtat ctaagtaccc gattagccct ggatagccc tggaacacgt tctgaaaccgt tataatgggt tataatgggt tataatgggt tccttgaacag tcgagcagga tcgagcagga ccgccagcta gctaggagg agtcggagg agtcggctgg	aaacagactt cttgtactga tgaggaaaag taagctcat cgtacgcga gaacatgggg atcgtgacga atcgcgaagg atgactta ttaataggc gttgaaggt atgacttgtg agctatttag ggcaatcccq agacacacgg aggtcatccaa tggcttagaagt	tgatcoggag atccataggt aaatcaacog tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg gtttagtcgc aggtaacacct gatcggaagg gtaggaaa cgggtaacacac gtagtagcctc acttaccaaa cgggtgctaa gtggggtgctaa gtggggct gtagacaccc gtaacggggc	atctctgaat gcaagaggg agattccctt ggaacgctct aggaacgctct ccaaggtaa aggacccgg cctacttgtt caagcttaac tgggtataga gactgaggataat atgtatcact ccgatgcaaa cgtcgtcgt agtggtaaac tcaagcaaa	120 180 240 300 360 420 480 540 660 720 780 840 900 960 1020 1140 1200
35 40 45	<213.5 Pseud <400 71 ggtcaagtag gtgaagtag gggggaacca aaccaggggg agtagtggc ggaagtggc gagtagtggc gagtagtggc gagtagtggc gatactaggac atactactactgagagggagt agtggagtgc ccgaacca ccgaaccac ccgaaccac ccgaaccaa caagtgggga ctggggggt ctcggaacca ccgaatac gaaaggggat agggggta ctgggggata cggaatac gaaaggggat gcgaatac gaaagggaatac gaaagggaatac agggaatac agggaagct agggaagct agggaagct agggaagct agggaagct agggaagct agggaagt	agaagcgcat aaaagcttcg acctaggata actgaaacagg gccatagtgg gccatagtgg gagaactgagc gagaatagaa gtaccttttg aggcgttagcg ggcgatctat ccagaagtgc ccagaactgt ccagaagtgc acaacccaga ggcttagac gccttagaca gcttagaca gccttagaca gccttagaca	acggtggatg gggagtcggc acctaggtat tchagtacc gattagccct gtgatagcct gtgatagcct ttgaaccagt ttgaaccagt tataaccagt tataaccagt tataaccgg tataatgggg tataatgggg tcccttgaacag tccgctagagcag cgacatggg ccgccagcta gctaggagtagcagcagcag	aaacagacttcat cttgtactga tgaggaaaag taagcttcat cgtacgcgaa gaacatgggg atgcgtacaa cagcgactta ttaatagggc ttgaaggtt atgacttaga ggtcatttag agctatttag agcaacccg aggtcccaaa aggcctaagag agggaagag ggggaagag ggggaagag ggggaagag	tgatcoggag atcataggt aaatcaaccg tgattttagc aggatctttg ggaccatcttg ggaccatcttg ggactatcatg gtttagtcgc aggtagag tattcagtgg gtttagtcgc aggtaacact gatagcgctc acttaccaaa gtgggctaa gtgtggttag gtagcgcct acttaccaaa gttgtggttag gtagcgctc acttaccaac gtgggctaa gtgttggtta agtgttggtta agcgctcc gtaacgggc agcgttctgt	atctctgaat gcaagaggg agattccctt ggaacgctct caagtgaaatc ccaaggctaa agaaccccgg cctacttgtt caagcttaac tgggtataga gactgaaga atgtatcact ccgatgcaaa cgtccgtcgt agtggtaaac tcaaaccaca aagcctgtga	120 180 240 360 420 480 540 660 720 780 960 1020 1080 1140 1200 1260
35 40 45	<213.9 Pseud 4400.7 1 99tcaagtga 9tagectagec 9g9ggaactgec aaccagggga aqtagtgac 9gsaagtgac aqtagtgac aqtagtgac aqtagtgac aqtggactga aqagggac ccgaaccac caagctcagga ctggagggac tcggagggac tcggagggac ccgaaccac aaacagggaa gogtaatag aaaggggaa gatgggggac aqtaggggac aqtaggggac aqtaggggac aqtaggggac aqtaggggac aqtagggac aqtagggac aqtagggac aqtagggac aqataggac aqataggac aqataggac aqataggac aqataggac aqataggac aqatagattg aqataggac aqatagattg aqataggattg	agaagcgcat aaaaagcttcg acctaggata actgaaacat agcgaacgg gccatagtg gcatagtg gagacagga ttgaccgata gaatagaac ttccgttga gagatctat tccgttga gafactctttc agaagctgc gcgatctat tccatagac gacactgt cagaagtgc acaatccaga ggctagac caatcagac gcgattga acaatcagac gcgagtgta agaagctgc agaagctgc agagcttgc agaagcttgc	acgytggatg gggagtcgg acctaggtat ctaagtatc ggattagccg aacttigtc gtgaaccagt ctgaaccgg tataatgggt aaggtaggag aaggtaggg gagcatggg ggagcatggg ggagcatggg ggtaggagg agtcggctg ggtaaggag tggaggtat tggaggtat agtcggctg	aaacagactt chtghactga tgaggaaaag taagcttcat cgtacgcga gaacatgggg atcgtgacga atcgcgacga atgcgtacaa cgttgaaggt ttgaattttg ggtcattcag ggtcattcag agcacacgg aggtcccaa tgcgttagaa cgcggaagat cgcggaagag agaagtscga aaccagggt	tgattcoggag aatcataggt aaatcaaccg tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggag tattcagtgg tattagtcgc aggtaacact gatcggaat gtagcgaca ggtggaaa gggtacaa aggggtgctaa gttgtggtta gcagcaccc gtaacggggc agcaccc tacgagag	atctctgaat gcaagaggg agattccctt ggaacgctct aagtgaaatc ccaaggttaa agaacccgg cctacttgtt caagcttaac tgggtataga gactggagga aaaggctaat atgtatcact ccgatgcaaa cgtccgtcgt agtggtaaaa cttaaagaaa atcaacaac aagcctgtga gagtaaacgac	120 180 240 300 360 420 540 660 720 780 840 900 1020 1080 1140 1200 1260 1320
35 40 45	<213.9 Pseud <400.5 71 ggtcastgs ggggggaacc ggaagtggcg ggaattgs ggatagtgc ggatagtgc ggatagtgc ggatagtgc ggatagtgc ggatagtgc ggatagtgc gaattgsggaagt ggaaagtgsagaagtgsggaagt ggaaggttagaaggttagaatgstgaatgsgaaggttagaatgsgaaggttagaaggtagaaggaaggtagaaggaaggtagaaggtagaaggtagaaggtagaaggtagaaggtagaaggtagaaggtagaagga	agaagcgcat aaaagcttcg acctaggata acctgaacat accgaacaggg gccatagtgg gccatagtgg gccatagtgg gagcacgaga traccttttg agagcgtagc ggcgatctat toccgttgaa gatagctgct agagcactgtt ccagaagtg accacccag gcgtagca gcctagaca tcactagaca tcactagaca cacaccag gcggtgtca gagagtgtca aaaacaccc	acgytgattg gggagtcgg acctaggtat ctaagtacc tctaagtacc gattagccc gattagccc gattagccc acctttgtct gtgaaaccqt ctgaaaccqt tataattggt tataattggt tataattgggt tataattgggt tataattgggt cccatgagcag caggtagcag cagcagtagg cagcatggg cagcagatgg cagcagcat gcagcagata gcagcagcat acgccgaaaa aggcagagc	aaacagactt chtgtactga tgaggaaaag taagcttcat cgtacgcgaa gacatgggg atcgtgacga atgcgtacaa gttgaaggt tagactttta gtcattcag agctattag agcaatcag aggcattagaa cgcggaagag gcggtagagg agaagagtgagag acaagggta	tgattcaggag aatcataggt aaatcaacag tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgggaa tattcagtgg tattagtcgc aggtaacact gatoggagt gtagcgccc acttaccaaa gtagcgctc acttaccaaa gtggtgctaa gttgtggtta gcggtacta gtagcgccc cactaccaac cggggctca cgcgcacc cctgaccacc cctgcgcaac cctgcgcaac	atctctgaat gcaagaggcg agattccctt aggaacgctct aaggaaccccgg cctaaggctaa agaaccccgg cctacttgtt aggatggaaaa tgggttaaa agactgaggaa aaggttaaa atgtatcact ccgatgcaa actgcgtcgt agtggtaaaa tttaaagaaa tttaaagaaa ttaaaccaca aagcctgtga gagtaacgac gagtaacgac gagtaacgac aagactgtga	120 180 240 300 360 420 540 660 720 780 840 900 900 1020 1140 1200 1230 1380
35 40 45 50	<213.9 Pseud 4400.7 1 99tcaagtga 9tagectagec 9g9ggaatgec aaccagggga aqtagtgage 9gsaagtgage 9gsaagtgage aqtagtgage ediactactga aqagggage ediactactga aqagggage ediacecae cagactegga eteggaggga eteggaggga eteggaggga eteggaggga aqtggaggga eteggaggga ediactage gaaatggga aqtggagga aqtggagga aqtggagga aqtggagga aqtggagga aqtggagga aqtggagga ediactage eaccagaaget aggatagttg aatggggttg aatgggttg aatgggttg aatgggttg aatgggttg aatgggttg aatgggttg aatggggttg aatggggttg	agaagcgcat aaaaagctteg acctaggata acctgaacat acgaacagg gcatagtgg gagcacgag cttgaccgtts gagcttagea ggcatctat gagcttagea ggcgatctat tcccgttgaa gagcatctgt agaccttgta ccagaagtg agacctgtt ccagaagtg accatagtcg gcgstctaaca ccagaagtg accatagtcg ccagagtgtca agaacttgtc ccagagtgtca agaacttgtc ccagagtgtca agaacttgtc ccagagtgtca agaagcttgc agaacttgtc ccagagttgc agacttgtc agaagcttgc agaacttgtc	acgytggatg gggagtcgg acctaggtat ctaagtacc ctaagtacc gtgtatagccc aactttgtct gtgaacacgt ctgaaaccgt tataatggg aaaggagtc ccatgagcag aaggtaggg ccgcagata gcagacatgg gcagcagta gcagagtagg gctaggagt gcggcagta gcggagtag gcggagtag gcggagtag agcggagtag agcggagtag agcggagtag agcggagtag agcggagtag	aaacagactt chtgtactga tgaggaaaag aacatgggg accgtgaggg accgtgaggg atcgtacaa cagcactta ttaataggg ttagaagst atgactteta ggccatttag ggcaatcccaa agcacaccg aggtcccaaa tggcttagaa cgcggaagat gcggaagag gagaagaggaga agaagtgcga aaccaagggt gagaagggt gagaagggt	tgattcaggag aatcataggt aaatcaacag tgattttagc aggatctttg ggaccatcct aaaggcgaaa gcagtgagag tattcagtgg gtttagtcgc aggtaacact gatggagtaacact gatggagtcacact gcgggtgctaa gttgtggtta gcagccacc gtaacaggggc tatgcggatgacacc gtaacggggc tatgcggatga	atctctgaat gcaagaggg agattcectt aggaacgtct aagtgaaatc ccaaggtta agaacecegg cctacttgtt caagttaac agaacecega acaggetaat agacecega acaggetaat acagetaat acagetaat acagetagaga atgtatgaaga tcaatgcaac tcttaagagaa tcaaaceac aagcetgtg agtgtaac gagtaacagac gttaategaa aacagttaa aagcttgcg aatgggaaac acaaggttaa aagcttgcgg aataggaa	120 180 240 300 360 420 480 540 660 720 780 840 900 900 1020 1140 1200 1260 1320 1380 1440
35 40 45	<213.5 Pseud <400.5 71 gytcasytga gytcasytga gytgagggaacc agyagggaacc aacagggga agyagggaacc gyaaagtgoc gyaaagtgoc gyaaagtgoc cogaaacca cogyaacacc cogaacacca cotyaacggg cocyaaacca cocyaacacca cocyaacacac cocyaacacaca agcttoga ctcogaacaca cocyaacacaca agcttoga ctcogaacaca cogaacacaca agcttoga ctcogaacaca agcttoga ctcogaacaca agcttoga ctcogaacaca agcttoga ctcogaacaca agaaagtyaga cocyaagt agytgaty agytgaty goaaggtta atccyatacac accyatacacacacacacacacacacacacacacacacac	agaagcgcat aaaagctcg acctaggsta acctgaacad accgaacaggg gccatagtgg gccatagtgg ggcacagaa ctgaccgata gagcgtagc ggcgatctat toccgttgaa gagcactgtt ccagaagtg agacactgtt ccagaagtg agcactgtt ccagaagtg accacccag ggcgtagca gccttagaca tcactaga	acgytgattg gggagtcgg acctaggtat ctaagtacc tctaagtacc gattagccc gattagccc gattagccc aactttgtct gtgaacacgt ctgaaaccgt tataattggt tataattggt tataattggt tataattggt tataattggt cccatgagcag cagcattggg cagcattggg cagcattggg cagcattggg cagcattggg cggcagtgg cggcagtgg cgcagaag agtcggccg cgtaagtga cgcagaag agcgaggc ctgcgatgag ctgcgatgag	aacagactt cttgtactga tgaggaaaag tgaggaaaag accgtgaag accgtgaag accgtgaag accgtgatag ttaataggg tttgaaggt ttaataggg tgtagaggt agcatcttgt agcatcttgt agcatcccg aggtcccaa tggctactag ggcagaaga ggcagaaga gcagaaga gcagaaga gagatgaga accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt accaagggt	tgatccggag aatcaacgg aatcaacgg aatcatcgg aggatcatct gggacatcct aaaggcgaa gcagtggag gtttagtcgc gtagcagcct actcacaac ggtagcaccc gtagcagccc gtagcgccc gtagcgccc gtagcgccc gtagcgccc gtagcgccc gtagcgccc gtagcgccc gtagcgagag ggcttctgt actcacaa gtgtggtag ggcgcacc gtagcgagag ggcgtccggaa ggcgtcagag ggcgtagagag aggctagagca aggcagaaca cctgcgaaa ggctagaga	atctctgaat gcaagaggcg agattccctt aggaacgctct aaggaaccccgg cctaaggctaa agaaccccgg cctacttgtt aggatggaaaa tgggttaaa agactgaggaa aaggttaaa atgtatcact ccgatgcaa actgcgtcgt agtggtaaaa tttaaagaaa tttaaagaaa ttaaaccaca aagcctgtga gagtaacgac gagtaacgac gagtaacgac aagactgtga	120 180 240 300 360 420 660 660 720 780 840 900 1020 1080 1140 1260 1320 1380 1380 1500

```
aagettetaa getteaggta accaggaace gtaccecaaa ccgacacagg tggtegggta 1620
     gagaatacca aggcgcttga gagaactcgg gtgaaggaac taggcaaaat ggcaccgtaa 1680
     cttcgggaga aggtgcgccg gctagggtga aggatttact ccgtaagctc tggctggtcg 1740
     aagataccag gccqctqcqa ctqtttatta aaaacacagc actctqcaaa cacqaaaqtq 1800
     qacqtataqq qtqtqacqcc tqcccqqtgc cgqaaqqtta attqatqqqg ttagcqcaaq 1860
     cqaaqctctt qatcqaaqcc ccgqtaaacg gcqqccgtaa ctataacgqt cctaaqgtag 1920
     cqaaatteet tgtegggtaa gtteegacet geacgaatgg egtaaegatg geggegetgt 1980
     ctccacccqa gactcaqtqa aattgaaatc gctgtgaaga tgcaqtgtat ccgcggctag 2040
     acggaaagac cccgtgaacc tttactgtag ctttgcactg gactttgagc ctgcttgtgt 2100
10 aggataggtg ggaggctttg aagcgtggac gccagttcgc gtggagccat ccttgaaata 2160
     ccaccetgge atgettgagg ttetaactet ggteegtaat ceggategag gacagtgtat 2220
     ggtgggcagt ttgactgggg cggtctcctc Ctaaagagta acggaggagt acgaaggtgc 2280
     gctcagaccg gtcggaaatc ggtcgcagag tataaaggca aaagcgcgct tgactgcgag 2340
     acagacacgt cgagcaggta cgaaagtagg tottagtgat ccggtggtto tgtatggaag 2400
15
    ggccatcgct caacggataa aaggtactcc ggggataaca ggctgatacc gcccaagagt 2460
     tcatatcgac ggcggtgttt ggcacctcga tgtcggctca tcacatcctg gggctgaagc 2520
     cqqtcccaaq ggtatggctg ttcgccattt aaagtggtac gcgagctggg tttagaacgt 2580
     cgtgagacag ttcggtccct atctgccgtg gacgtttgag atttgagagg ggctgctcct 2640
     agtacgagag gaccggagtg gacgaacete tggtgttccg gttgtcacge cagtggcatt 2700
20
     gccqqqtaqc tatqttcqqa aaaqataacc qctqaaaqca tctaaqcqqq aaacttqcct 2760
     caagatgaga totcactggg aacttgatto cootgaaggg cogtogaaga ctacgacgtt 2820
     gataggetgg gtgtgtaage gttgtgagge gttgagetaa ccagtactaa ttgcccgtga 2880
     qqcttqacca t
25
     <210> 72
     <211> 2886
     <212> DNA
     <213> Vibrio cholerae
30
     <400> 72
     ggttaagtga ctaagcgtac acggtggatg cctgggcagt cagaggcgat gaaggacgta 60
     ctaacttgcg ataagcgcag ataaggcagt aagagccgtt tgagtctgcg atttccgaat 120
     ggggaaaccc aactgcataa gcagttactg ttaactgaat acataggtta acagagcaaa 180
35
     ccqqqqqaac tqaaacatct aaqtaccccq aggagaagaa atcaaccgag attccggtag 240
     tagoggogag cqaacctgga ttagocotta agcactoggt qaagtaggtg aacaagctgg 300.
     aaagettgge gatacagggt gatageeeg taacegaege tteategage gtgaaatega 360
     gtagggcggg acacgtgata teetgtetga atatgggggg accatectee aaggetaaat 420
    actectgact gaccgatagt gaaccagtac cgtgaggaaa ggcgaaaaga acccctgtga 480
40
     ggggagtgaa atagaacctg aaaccgtgta cgtacaagca gtaggagcac cttcgtggtg 540
     tgactgcgta ccttttgtat aatgggtcag cgacttatat tcagtggcaa ggttaaccgt 600
     atagggage cqtaqeqaaa qeqaqtetta actqqqeqet caqtetetqq atataqacec 660
     qaaaccqqqt qatctaqcca tqqqcaqqtt qaaqqttqaq taacatcaac tqqaqqaccq 720
     aaccqactaa tqttqaaaaa ttaqcqqatq acttqtqqct aqqqqtqaaa qqccaatcaa 780
45
     actoggagat agotgqttct coccgaaagc tatttaggta gcgcctcgga cgaatactac 840
     tqqqqqtaqa qcactqttaa qqctaqqqqq tcatcccqac ttaccaaccc tttgcaaact 900
     ccqaatacca qtaaqtacta tccqqqaqac acacggcqqq tgctaacgtc cgtcgtqqaq 960
     agggaaacaa cccagaccgc cagctaaggt cccaaagtat tgctaagtgg gaaacgatgt 1020
     qqqaaqqctc agacagctag gatgttggct taqaaqcaqc catcatttaa agaaagcgta 1080
50
    atageteact agtegagteg geetgegegg aagatgtaac ggggetaage aatacacega 1140
     agctgcggca atatctttta gatattgggt aggggagcgt tctgtaagcc gttgaaggtg 1200
     aatcqtaaqq tttqctqqaq qtatcaqaaq tqcqaatqct qacatqaqta acqacaaaqq 1260
    gggtgaaaaa cctcctcgcc ggaagaccaa gggttcctgt ccaacgttaa tcggggcagg 1320
    gtgagtcgac ccctaaggtg aggccgaaag gcgtaatcga tgggaaacgg gttaatattc 1380
55
    cogtacttot gactattgcg atggggggc ggagaaggct aggtgggcca ggcgacggtt 1440
    gtcctggttc aagtgcgtag gcttgagagt taggtaaatc cggctctctc taaggctgag 1500
```

```
acacgacqtc gagctactac ggtagtgaag tcattgatgc catgcttcca ggaaaaqcct 1560
     ctaagettea gatagteagg aategtacee caaacegaca caggtggteg ggtagagaat 1620
     accaaggege ttgagagaac tegggtgaag gaactaggea aaatggtace gtaacttegg 1680
     gagaaggtac gctcttgatg gtgaagtccc tcgcggatgg agctgacgag agtcgcagat 1740
   accappinge typeactytt tattaaaaac acagcactyt gcaaaatcyc aagatyacyt 1800
     atacggtgtg acgcctgccc ggtgccggaa ggttaattga tggggttagc gcaagcgaag 1860
     ctcttgatcg aagccccggt aaacggcggc cgtaactata acggtcctaa ggtagcgaaa 1920
     tteettqteq qqtaaqttee qacetqcacq aatqqcqtaa tqatqqccac qctqtetcca 1980
     cccqagactc agtqaaattq aaatcqctqt qaaqatqcaq tqtacccqcq qctaqacqqa 2040
10 aagaccccgt gaacctttac tacagcttgg cactgaacat tgaacctaca tgtgtaggat 2100
     aggtgggagg ctatgaagac gtgacgccag ttgcgttgga gccgtccttg aaataccacc 2160
    cttgtatgtt tgatgttcta acttagaccc gttatccggg ttgaggacag tgcctggtgg 2220
     gtagtttgac tggggcggtc tcctcccaaa gagtaacgga ggagcacgaa qgtgggctaa 2280
    tcacggttgg acatcgtgag gttagtgcaa tggcataagc ccgcttaact gcgagaatqa 2340
15
   cggttcgagc aggtgcgaaa gcaggtcata gtgatccggt ggttctgtat ggaagggcca 2400
     togotcaacg gataaaaggt actcogggga taacaggotg ataccgccca agagttcata 2460
     tegacggegg tgtttggcac ctegatgteg gctcatcaca teetgggget gaagteggte 2520
     ccaagggtat ggctgttcgc catttaaagt ggtacgcgag ctgggtttag aacgtcgtga 2580
     gacaqttegg tecetatetg cegtgggegt tggaaqattg aagggggetq etectaqtac 2640
20
    gagaggaccg gagtggacga acctctggtg ttcgggttgt gtcgccagac gcattgcccg 2700
     gtagctaagt teggaattga taagegetga aageatetaa gegegaageg ageeetgaga 2760
     tgagtottoc otgacagttt aactgtocta aagggttgtt ogagactaga acgttgatag 2820
     gcagggtgtg taagcgttgt gaggcgttga gctaacctgt actaattgcc cgtgaggctt 2880
     aaccat
25
     <210> 73
     <211> 2906
     <212> DNA
30
     <213> Yersinia enterocolitica
     -2205
     <221> modified_base
    <222> (1168)..(1178)
35
    <223> N = A, C, G or T/U
    <400> 73
    ggttaagcga ccaagcgtac acggtggatg cctaggcagt cagaggcgat gaaggacgtg 60
    Ctaatctgcg aaaagcgtcg gtaaggtgat atgaaccgtt ataaccgacg atacccgaat 120
    ggggaaaccc agtgcaattc gttgcactat tgcatggtga atacatagcc atgcaaggcg 180
     aaccggggga actgaaacat ctaagtaccc cgaggaaaag aaatcaaccg agattcccc 240
    agtagoggeg agogaacggg gaggagccca gaacctgaat cagcgtatgt gttagtggaa 300
    gcgtctggaa agtcgcacgg tacagggtga tagtcccgta cacaaaaatg catatgttgt 360
    gagttegatg agtagggegg gacacgtgac atcetgtetg aatatggggg gaccatecte 420
    caaggctaaa tactcctgac tgaccgatag tgaaccagta ccgtgaggga aaggcgaaaa 480
    qaaccccqqc qaqqqaqtq aaacaqaacc tqaaaccqtq tacqtacaaq caqtqqqaqc 540
     acctteding totgacting tacctttint ataatoggte agggacttat atttingtage 600
     aaggttaacc gaatagggga gccgtaggga aaccgagtct taactgggcg aatagttgca 660
    aggtatagac ccgaaacccg gtgatctagc catgggcagg ttgaaggttg ggtaacacta 720
    actggaggac cgaaccgact aatgttgaaa aattagcgga tgacttgtgg ctgggggtga 780
    aaggccaatc aaaccgggag atagctggtt ctccccgaaa gctatttagg tagcgcctcg 840
    tgaactcatc ttcgggggta gagcactgtt tcggctaggg ggtcatcccg acttaccaaa 900
    ccgatgcaaa ctccgaatac cgaagaatgt tatcacggga gacacacggc gggtgctaac 960
    gtccgtcgtg aagagggaaa caacccagac cgccagctaa qqtcccaaaag tcatqqttaa 1020
55
    gtgggaaacg atgtgggaag gcacagacag ccaggatgtt ggcttagaag cagccatcat 1080
    ttaaagaaag cgtaatagct cactggtcga gtcggcctgc gcggaagatq taacggggct 1140
```

```
aaaccatqca ccgaaqctqc qqcaqcqnnn nnnnnnnnn nnnnnnnnqq qqaqcqttct 1200
     qtaaqccqtt qaaqqtqacc tqtqaqqqtt qctqqaqqta tcaqaaqtqc qaatqctqac 1260
     ataaqtaacq ataatqcqqq tqaaaaaccc qcacqccqqa aqaccaaqqq ttcctqtcca 1320
     acqttaatcq qqqcaqqqtq aqtcqacccc taaqqcqaqq ctqaaaqqcq taqtcqatqq 1380
     qaaacaqqtt aatattcctq tacttqqtqt tactqcqaaq qqqqqacqqa qaaqqctatq 1440
     ctagcogggc gacggttqtc ccqqtttaaq catqtaqqcq qaqtqaccaq qtaaatccqq 1500
     ttgcttatca acqctqaqgt qtqatqacqa qtcactacqq tqatqaaqta gttqatqcca 1560
     tgcttccagg aaaagcctct aagcatcagg taacatgaaa tcgtacccca aaccgacaca 1620
     ggtggtcagg tagagaatac tcaggcgctt gagagaactc gggtgaagga actaggcaaa 1680
10 atoptgccgt aacttcggga gaaggcacgc tgacacgtag gtgaagcggt ttacccgtgg 1740
     agctgaagtc agtcgaagat accagctggc tgcaactgtt tattaaaaac acagcactgt 1800
     gcaaacacga aagtggacgt atacggtgtg acgcctgccc ggtgctggaa ggttaattga 1860
     tggggtcagc gcaagcgaag ctcttgatcg aagccccggt aaacggcggc cgtaactata 1920
     acggtcctaa ggtagcgaaa ttccttgtcg ggtaagttcc gacctgcacg aatggcgtaa 1980
     tgatggccag gctgtctcca cccgagactc agtgaaattg aactcgctgt gaagatgcag 2040
15
     tgtacccgcg gcaagacgga aagaccccgt gaacctttac tatagcttga cactgaacat 2100
     tgagccttga tgtgtaggat aggtgggagg cataqaagtg tggacqccag tctgcatgga 2160
     gccaaccttg aaataccacc ctttaatgtt tgatgttcta acteggcccc gtaatccggg 2220
     gtgaggacag tgtcaggtgg gtagtttgac tgggggggtc tcctcccaaa gagtaacgga 2280
     qqaqcacqaa qqttaqctaa tcacqqtcqq acatcqtqaq qttaqtqcaa agqcataaqc 2340
     tagetteact gegagagtga eggetegage aggtaegaaa gtaggtetta gtgateeggt 2400
     ggttctgaat ggaagggcca tcgctcaacg gataaaaggt actccgggga taacaggctg 2460
     ataccgccca agagttcata tcgacggcgg tgtttggcac ctcgatgtcg gctcatcaca 2520
     tcctggggct gaagtaggtc ccaagggtat ggctgttcgc catttaaagt ggtacgcgag 2580
25
     ctgggtttag aacgtcgtga gacagttcgg tccctatctg ccgtgggcgy tggarraytg 2640
     agragageta etectaqtac gagaggaccg gagtggacgm atcactggtg ttcqqqttqt 2700
     catgccaatg gcaytgcccg gtagctaaat koggaagaga taasygctga aagcatctaa 2760
     gersgaaact tgccycgaga tgagttetee etgagactae aagteteetg aaggaacgtt 2820
     gaagacgacg acgttgatag gcygggtgtg taagcgcgag ttggcgttga gctaaccggt 2880
30
     actaatgaac cotgaggett aacett
     <210> 74
     <211> 23
35
     <212> DNA
     <213> Artificial Seguence
     <223> Description of Artificial Sequence: Synthetic
40
           Primer
     <400> 74
     gggttgeget egttaeggga ett
                                                                       23
45
     <210> 75
     <211> 23
     <212> DNA
     <213> Artificial Sequence
50
     <223> Description of Artificial Sequence: Synthetic
           Primer
55
    <400> 75
     gggttgcgct cgttgccgga ctt
                                                                       23
```

	<210> 76	
	<211> 23	
5	<212> DNA	
,	<213> Artificial Sequence	
	<213> Artificial Sequence	
	<220>	
	<223> Description of Artificial Sequence: Synthetic	
10	Primer	
	<400> 76	
	tececactge tgeeteeegt agg	23
15		
	<210> 77	
	<211> 23	
	<212> DNA	
	<213> Artificial Sequence	
20	Table Table Degrades	
	<220>	
	<223> Description of Artificial Sequence: Synthetic	
	Primer	
	Primer	
25		
23	<400> 77	
	caacatctca cgacacgagc tga	23
	<210> 78	
30	<211> 23	
	<212> DNA	
	<213> Artificial Sequence	
	<220>	
35	<223> Description of Artificial Sequence: Synthetic	
	Primer	
	<400> 78	
	tccccactgc tgcctcccgt agg	23
40	the second secon	2.5
	<210> 79	
	<211> 22	
	<211> 22 <212> DNA	
45		
43	<213> Artificial Sequence	

	<220>	
	<223> Description of Artificial Sequence: Synthetic	
	Primer	
50	·	
	<400> 79	
	ttaccgcggc tgctggcacg ga	22
55	<210> 80	
	<211> 23	

	<212> DNA		
	<213> Artificial Sequence		
	•		
	<220>		
5	<223> Description of Artificial Sequence:	Synthetic	
	Primer		
	<400> 80		23
10	ccccgtcaat tcctttgagt ttc		23
10			
	<210> 81		
	<211> 23		
	<212> DNA		
15	<213> Artificial Sequence		
	<220>		
	<223> Description of Artificial Sequence: Primer	Synthetic	
20	Primer		
20	<400> 81		
	caacatctca cgacacgagc tga		23
25	<210> 82		
	<211> 23		
	<212> DNA <213> Artificial Sequence		
	<213> Artificial Sequence		
30	<220>		
	<223> Description of Artificial Sequence:	Synthetic	
	Primer	-	
26	<400> 82		
35	tttcaccttt ccctcacggt act		23
	<210> 83		
	<211> 23	•	
40	<212> DNA		
	<213> Artificial Sequence		
	2.2		
	<220>		
45	<223> Description of Artificial Sequence: Primer	Synthetic	
73	FIIMEL		
	<400> 83		
	ggttetttte acettteeet ege		23
50			
	<210> 84		
	<211> 23		
	<212> DNA		
55	<213> Artificial Sequence		
,,	<220>		

	<223> Description of Artificial Sequence: Primer	Synthetic	
5	<400> 84 tggtttcagg ttctatttca ctc		23
10	<210> 85 <211> 22 <212> DNA <213> Artificial Sequence		
15	<220> <223> Description of Artificial Sequence: Primer	Synthetic	
	<400> 85 tttaaccgac aaggaatttc gc		22
20	<210> 86 <211> 23 <212> DNA		
25	<213> Artificial Sequence <220> <223> Description of Artificial Sequence: Primer	Synthetic	
30	<400> 86 ggttctttc accttccct cgc		23
35	<210> 87 <211> 15 <212> DNA <213> Artificial Sequence		
40	<220> <223> Description of Artificial Sequence: Primer	Synthetic	
45	<400> 87 taacctggtc gtaac		15
50	<210> 88 <211> 14 <212> DNA <213> Artificial Sequence		
55	<220> <223> Description of Artificial Sequence: Primer	Synthetic	
-	-400- 88		

	ecceccec ecce	14
5	<210> 89 <211> 16 <212> DNA <213> Artificial Sequence	
10	<220> <223> Description of Artificial Sequence: Synthetic Primer	
15	<400> 89 gcccctaacc tcgtcg	16
20	<210> 90 <211> 26 <212> DNA <213> Artificial Sequence	
25	<220> <223> Description of Artificial Sequence: Synthetic Primer	
	<400> 90 eggeectage egggtegtac etcegg	26
30	<210> 91 <211> 26 <212> DNA <213> Drifficial Sequence	
35	<220> <223> Description of Artificial Sequence: Synthetic Primer	
40	<400> 91 cggccctaac ctggtcgtaa ctccgg	26
45	<210> 92 <211> 23 <212> DNA <213> Artificial Sequence	
50	<220> <223> Description of Artificial Sequence: Synthetic Primer	
	<400> 92 aggettegat ceegggatee geg	23